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Role of Filamentous Phage SW1 in Regulating the Lateral Flagella of *Shewanella piezotolerans* Strain WP3 at Low Temperatures

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Low-temperature ecosystems represent the largest biosphere on Earth, and yet our understanding of the roles of bacteriophages in these systems is limited. Here, the influence of the cold-active filamentous phage SW1 on the phenotype and gene transcription of its host, *Shewanella piezotolerans* WP3 (WP3), was investigated by construction of a phage-free strain (WP3ΔSW1), which was compared with the wild-type strain. The expression of 49 genes, including 16 lateral flagellar genes, was found to be significantly influenced by SW1 at 4°C, as demonstrated by comparative whole-genome microarray analysis. WP3ΔSW1 was shown to have a higher production of lateral flagella than WP3 and enhanced swarming motility when cultivated on solid agar plates. Besides, SW1 has a remarkable impact on the expression of a variety of host genes in liquid culture, particularly the genes related to the membrane and to the production of lateral flagella. These results suggest that the deep-sea bacterium WP3 might balance the high-energy demands of phage maintenance and swarming motility at low temperatures. The phage SW1 is shown to have a significant influence on the swarming ability of the host and thus may play an important role in adjusting the fitness of the cells in the deep-sea environment.

Due to a growing awareness that viruses, especially phages, are the most abundant biological entities on the planet and that they play an irreplaceable role in all types of ecosystems, research into phage-host interactions has garnered much attention. Cold environments, including glaciers, permafrost, and deep seas, cover a large part of the earth, and viruses are abundant and functional in these environments (1–4). Nevertheless, our understanding of phage-host interactions and of the extent to which phages influence the adaptation and evolution of hosts in low-temperature environments is still incomplete (4).

It is well known that the production and life cycles of temperate phages are under the strict control of the host (5–8). In recent years, it has become increasingly apparent that temperate phages could modulate the functions of host cells. In *Escherichia coli*, phages have been found to influence divergent functions of the lysogenic cell, including replication, transcription, translation, degradation, and proteolysis (9–13). Bacterial viruses can regulate the expression of host genes and decrease the growth rate of *E. coli* in energy-poor environments, thus increasing the population fitness of *E. coli* (14). Although they can be cryptic and lose their basic function during evolution, prophages allow bacteria to cope with adverse environments and osmotic, oxidative, and acid stresses, increasing host growth and biofilm formation (15, 16).

The filamentous phages are temperate phages whose DNA integrates into the bacterial genome, and they become prophages and replicate with the host (17). In addition, some of the phage DNA can exist in the cytoplasm, such as in a plasmid, and this is called replicative-form (RF) DNA. The release of filamentous virions does not lead to lysis of the bacterial host. The outer membrane protein pIV multimer was found to form a barrel-like channel and was used for the elongating of Ff phage (18); however, some other filamentous phages utilize secretins of the host type II secretion system or type IV pilus system (19, 20) rather than encoding the outer membrane channel for phage particle secretion by themselves. CTXΦ, which infects *Vibrio cholerae*, is one of the most thoroughly investigated filamentous phages. As a consequence of carrying the *ctxAB* genes, which encode the cholera toxin, CTXΦ phages are beneficial for the multiplication and dissemination of *V. cholerae*. Moreover, CTXΦ is the key factor for the evolution of its bacterial host pathogenicity (5). Transcriptomic comparison of M13-infected and uninfected *E. coli* bacteria demonstrated that the oxidative and glutamate-dependent acid resistance systems are significantly affected by the phage (9). The filamentous prophage Pf4 has been shown to be a major contributor to the biofilm life cycle and the virulence of *Pseudomonas aeruginosa* (21).

As the first filamentous phage isolated from the deep-sea environment, SW1 possesses a genomic organization similar to that of other typical filamentous phages such as M13 and CTXΦ (22). The host of SW1 is *Shewanella piezotolerans* WP3 (here referred to as WP3), which was isolated from West Pacific sediment at a depth of 1,914 m, an environment with permanent low temperatures of approximately 2 to 4°C. The optimal growth temperature and pressure of WP3 are 20°C and 20 MPa, respectively (23, 24). Our previous research revealed that SW1 was significantly induced at a low temperature (4°C), in terms of phage DNA replication, gene transcription, and phage particle production (22, 25). Here, we used the cold-active SW1 phage and its WP3 host to investigate the influence of phages on the gene transcription and growth of the host strain at low temperatures. Phage SW1 was shown to have a

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TABLE 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Reference or source
E. coli strain		
WM3064	Donor strain for conjugation; Δ*dapA*	27
S. piezotolerans WP3 strains		
WP3	Wild type; GenBank accession no. CP000472	Laboratory stock
WP3ΔSW1-PP	WP3, deletion mutant of SW1 prophage	This work
WP3ΔSW1	WP3, deletion mutant of SW1 prophage and RF DNA	This work
Plasmids		
pRE112	Allelic-exchange vector; Cm^r *sacB*	28
pRE112-SW1	pRE112 containing the PCR fragment for deleting SW1 prophage	This work

TABLE 2 Primers and probe used in this study

Primer name	Sequence (5′–3′)	Description
SW1UL	AATTGAGCTCAGCACCTTATGGGCAGTC	SW1 deletion
SW1UR	TAAGGGCACCATATCGGCATTTGTTCGTC	SW1 deletion
SW1DL	AAATGCCGATATGGTGCCCTTAGGTATTT	SW1 deletion
SW1DR	GCCGTCTAGAACGGCTTTGTAGAAGATGG	SW1 deletion
SW1ΔconfirmFor	TTTACCAGAGGTCGCAGAA	Mutant confirmation
SW1ΔconfirmRev	CAGCCAAGTATAGTCACCAATT	Mutant confirmation
Chlfor	TAAATACCTGTGACGGAAGAT	Mutant confirmation
Chlrev	TATCACTTATTCAGGCGTAGC	Mutant confirmation
ORF116For	ATGACAATTGGTGAAAATATAAAG	Mutant confirmation
ORF116Rev	TTAACCCTGTTTTTTCTTTTCTTT	Mutant confirmation
lafARTFor	AAACAGCCAGCCGTAACGTT	qPCR
lafARTRev	TGCACCATCTGCAGTTTGGA	qPCR
fliA2RTFor	TTTTGGCCATCGAAGACATG	qPCR
fliA2RTRev	GCCTTGCGGACTCGAGTAAC	qPCR
swp0265RTFor	TGGCGAGCATGTCACTACAGA	qPCR
swp0265RTRev	GGGCTGATTTGCCATCCA	qPCR
swp3209RTFor	GGTGAGTTCAACGGCAAAGG	qPCR
swp3209RTRev	CGGTGTCATGGTACTCTTGTTTG	qPCR
swp4513RTFor	TGACAGCAAATACGCCAACCT	qPCR
swp4513RTrev	CCCATCACATATGCAGCGTTT	qPCR
swp0958RTFor	CATTGCAGCCAGTGATTTGG	qPCR
swp0958RTRev	GAAGGGCGCTGATGGATCT	qPCR
swp1364RTFor	TTGCGTACCGCACGAGAA	qPCR
swp1364RTRev	TCCGCTGTCGGTTCATGAT	qPCR
pepNRTFor	TTAAGGCAATGGAAGCTGCAT	qPCR
pepNRTRev	CGTCTTTACCCGTTAATGATACGA	qPCR
SW1 minus probe[a]	TTACGCTTTGTTAACTGCACGCTTTG CAATCGTAATACCCTTAAGCGCAAG GTTAATACCAACAATCAGAACACCT GTACCAGCAATGAAAGTTGCAACA	Southern blot

[a] Genome location positions 2691372–2691471.

significant impact on the expression of 49 genes of the host cell, particularly genes for lateral flagellum production, and thus influences the swarming ability of the host at 4°C. Considering the importance of swarming motility for the surface movement and nutrient harvesting of bacteria on deep-sea sediments (26), the SW1 phage may make a significant contribution to the fitness of the bacterium in deep-sea environments.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and growth assay. All bacterial strains and plasmids used in this study are listed in Table 1. The *Shewanella* strains were cultured in modified 2216E marine medium (2216E) (5 g/liter tryptone, 1 g/liter yeast extract, 0.1 g/liter $FePO_4$, 34 g/liter NaCl) with shaking at 220 rpm at different temperatures. *E. coli* strain WM3064 was incubated in lysogeny broth (LB) medium (10 g/liter tryptone, 5 g/liter yeast extract, 10 g/liter NaCl) at 37°C with the addition of 50 μg/ml DL-α,ε-diaminopimelic acid (DAP). For solid medium, agar (Sangon Inc., Shanghai, China) was added at 1.5% (wt/vol). The antibiotic chloramphenicol (Cm) (Sigma, St. Louis, MO) was added to the medium at 25 μg/ml and 12.5 μg/ml for *E. coli* and *Shewanella* strains, respectively, when required. The growth of the WP3 strains was determined using turbidity measurements at 600 nm with 2216E.

Construction of a phage-free mutant. A SW1 prophage deletion mutant was constructed as described previously (29). First, the upstream and downstream fragments flanking both sides of SW1 prophage were amplified with the PCR primer pairs (Table 2). These two fragments were used as templates in a second fusion PCR, resulting in a fragment with a deletion in the SW1 prophage gene. Then, the PCR product was cloned into pRE112 as a SacI-XbaI fragment, yielding pRE112-SW1. This plasmid was transformed into *E. coli* WM3064 and then into WP3 by two-parent conjugation. The transconjugant was selected by chloramphenicol resistance and verified by PCR. The WP3 strain with pRE112-SW1 inserted into the chromosome was plated on 2216E agar medium supplemented with 10% sucrose. A successful prophage deletion mutant was screened and confirmed by PCR. The RF DNA of SW1 was cleared by methods previously described (30) with some modifications. In brief, WP3ΔSW1-PP was incubated in 5 ml of 2216E broth supplemented with 0.025% SDS for 24 h at 20°C, and then 100 μl of the bacterial culture was transferred to 5 ml of fresh medium and incubated for 24 h at 28°C. After every 5 rounds of this temperature-alternating culture procedure, the SW1 RF DNA removal was checked by PCR, and a SW1-free WP3 strain was screened out and confirmed after 30 rounds of cultivation. This mutant was designated WP3ΔSW1 (Table 1).

RNA isolation and real-time qPCR. The WP3 and WP3ΔSW1 strains were cultured in 2216E medium at 4°C, and the culture was collected immediately when the cells reached mid-exponential phase (optical density at 600 nm [OD_{600}] = ~0.8). Total RNA extraction, reverse transcription, and real-time quantitative PCR (qPCR) were performed as described previously (29, 31). The primer pairs (Table 2) used to amplify the selected genes in qPCR were designed using Primer Express software (Applied Biosystems).

DNA microarray construction. PCR primers for 4,744 of the 4,945 predicted open reading frames (ORFs) in the WP3 genome (excluding 200 coding sequences [CDSs] shorter than 150 bp) were designed using Primer version 5.0 and then synthesized (BioAsia Biotech, Shanghai, China). The following criteria were used to identify the optimal forward and reverse primers to generate PCR products specific for each selected ORF: (i) the entire ORF was used as a probe in cases in which it was <75% similar to all other genes in the genome; (ii) for homologous genes, the maximal portions of the genes showing <75% similarity were selected as specific probes; (iii) for homologous genes where no specific fragments could be identified, one of the genes was selected as a probe to represent the entire gene group; and (iv) each oligonucleotide primer contained 20 to 25 bases. To simplify the PCR amplification, most of the primer sets were designed to have annealing temperatures of ~60°C. ORF-specific fragments were amplified by *Taq* DNA polymerase with the following cycling conditions: denaturation for 30 s at 95°C, annealing for 1 min at 60°C, and extension for 1.5 min at 72°C, along with an initial 5-min denaturation at 95°C and a final extension for 10 min at 72°C. All PCR

products were purified using ethanol precipitation. The quality of the amplified products was checked by 1.5% agarose gel electrophoresis and ethidium bromide staining. Amplified DNA fragments were considered correct if the PCR results contained a single product of the expected size. The PCR for 94 genes consistently yielded unsatisfactory results with respect to products (e.g., no product, a product of the wrong size, of multiple or faint bands). Of the 4,744 genes with designed primers, 4,650 ORFs were correctly amplified. We used specific 70-mer oligonucleotides to represent the 39 ORFs which were not successfully amplified. In total, the PCR amplicons and oligonucleotide probes represented 95% of the total predicted gene content of WP3. The PCR products and microarray reagents were arrayed from 384-well microtiter probes printed in triplicate onto Telechem Superamine slides (Telechem, Sunnyvale, CA). The printed slides were dried and subjected to UV cross-linking.

Preparation of fluorescent dye-labeled DNA and hybridizations. The total RNAs were reverse transcribed with SuperScript II (Invitrogen, Carlsbad, CA), and the cDNAs were labeled with Cy3 and Cy5 by using the Klenow enzyme (TaKaRa Bio Inc., Japan) according to the manufacturers' instructions. Labeled cDNA was purified with a PCR purification kit (Macherey-Nagel, Düren, Germany) and resuspended in elution buffer. Labeled controls and test samples were quantitatively adjusted based on the efficiency of the Cy dye incorporation and mixed with 30 μl of hybridization solution (50% formamide, 1× hybridization buffer; Amersham Biosciences). The DNA in the hybridization solution was denatured at 95°C for 3 min prior to loading onto a microarray. The arrays were hybridized overnight at 42°C and washed with 2 consecutive solutions (0.2% SDS–2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] for 5 min at 42°C and 0.2× SSC for 5 min at room temperature [RT]). The microarray slides were hybridized with cDNA prepared from 3 biological replicate samples. As a measure of technical replication, the dye-swap experiment was performed on each sample such that a total of 6 data points were available for every ORF on the microarrays.

Image acquisition, data processing, and clustering. A LuxScan 10K scanner and microarray scanner 2.3 software (CapitalBio, Beijing, China) were used for the array image acquisition. We quantified the signal intensities of individual spots from the 24-bit TIFF images using SpotData Pro 2.2 (CapitalBio, Beijing, China). The linear normalization method, based on the expression levels of WP3 housekeeping genes in combination with the yeast external controls, was used for data analysis. The normalized data were log transformed and loaded into MAANOVA under the R environment for multiple testing by fitting a mixed-effects analysis of variance (ANOVA) model (32). Microarray spots with P values < 0.001 in the F-test were regarded as differentially expressed genes (DEGs). In addition, all of the DEGs were confirmed with Significance Analysis of Microarrays (SAM) software (33).

Southern blot. WP3 chromosomal DNA was electrophoresed on a 0.8% (wt/vol) agarose gel after digestion with SacI and BamHI overnight at 37°C and was then transferred to a nylon Hybond-N+ membrane (Amersham Biosciences). The SW1 minus-strand probe (Sangon, Shanghai, China) which corresponds to the major coat protein encoded by SW1 prophage gene (swp2565) was labeled with digoxigenin (DIG) using a DIG DNA labeling and detection kit (Roche, Mannheim, Germany) according to the manufacturers' instructions.

Motility assay and transmission electron microscopy (TEM). For motility assays, single colonies from overnight streaked plates were placed on the swimming plates (2216E medium with 0.3% agar; Eiken Chemical, Tokyo, Japan) and swarming plates (2216E medium with 0.7% agar) with sterilized toothpicks. For the swimming motility assay, the plates were incubated at 20°C and 4°C for 3 days and 7 days, respectively. For the swarming motility assay, the plates were incubated at 20°C and 4°C for 4 days and 14 days, respectively. The motility was assessed by examining the migration distance of the bacteria from one side to another side of the colony edge (Maximal swarming distance). For each strain, the assays were performed on at least four different days with three independent cultures spotted onto three plates each day. The data were analyzed by Student's two-tailed t test using Excel software (Microsoft Corporation). For transmission electron microscopy (TEM), bacteria were grown on the swarming agar plates and suspended in 1% (wt/vol) sterile NaCl solution. The samples were placed onto a carbon-coated grid (200 mesh) and stained with 0.5% phosphotungstic acid (PTA). The grid was air dried and examined under a Tecnai G2 Spirit Biotwin microscope (FEI Company, Eindhoven, Netherlands).

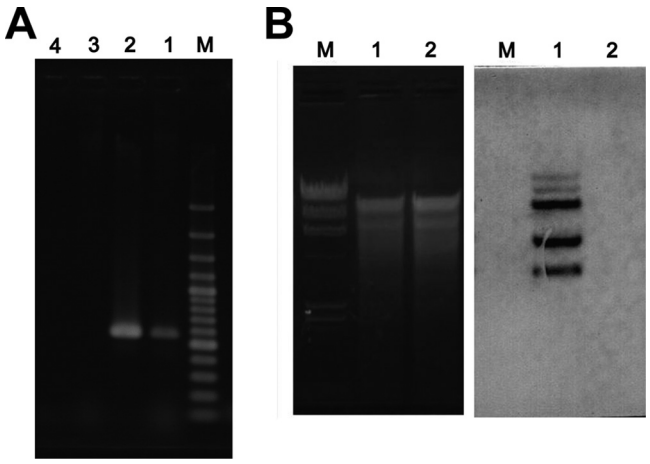

FIG 1 PCR (A) and Southern blot (B) confirmation of the phage-free WP3 strain. (A) Lanes: M, 100-bp DNA ladder; 1, WP3ΔSW1-PP DNA; 2, WP3 DNA; 3, WP3ΔSW1 DNA; 4, negative control. (B) Lanes: M, lambda DNA/HindIII marker; 1, WP3 DNA; 2, WP3ΔSW1 DNA. The genomic DNA was digested with SacI and BamHI before electrophoresis. Left panel, agarose gel electrophoresis of enzyme-digested total DNA. Right panel, Southern blot results determined by using the gel shown to the left.

Microarray data accession number. The microarray data have been deposited in the NCBI Gene Expression Omnibus (GEO) and are accessible through GEO series accession number GSE43882.

RESULTS

Construction of the phage-free WP3 strain and growth assay. Because WP3 contains an integrated prophage within its genome and RF DNA in the cytoplasm, the phage-free strain was constructed by two steps. First, the 8-kb fragment containing the intact SW1 prophage was deleted from the WP3 genome, and the phage-defective WP3ΔSW1-PP strain was constructed. WP3ΔSW1-PP was further treated with SDS and cultured at alternating temperatures to eliminate the SW1 RF DNA. Finally, the phage-free WP3ΔSW1 strain was constructed successfully. Both PCR and Southern blot hybridization confirmed the clearance of SW1 from WP3 (Fig. 1).

Is the virus-host relationship a burden or a benefit to the host population (34)? The key to answering this question is understanding how the host balances the costs and benefits of keeping the viruses. Temperate phages could confer not only host immunity to other phages but also fitness (9, 13–16, 21, 35). Low temperature is one of the primary obstacles facing microorganisms. Here, the possible impact of SW1 on the growth of WP3 was initially investigated at different temperatures (see Fig. S1 in the supplemental material). SW1 was not observed to influence the growth of WP3 when the strains were cultivated either at 20°C (the optimal growth temperature of WP3) or at 4°C, whether the phage was silent (20°C) or active (4°C) (22).

SW1 influences the gene transcription of host cells. Considering the fact that SW1 is metabolically active at 4°C, whole-genome microarray analysis was performed to identify possible in-

TABLE 3 Differentially expressed genes in WP3ΔSW1 at 4°C[a]

Functional category and gene locus designation	Avg fold change (WP3ΔSW1/WP3)	Product and description	COG designation or no. of relevant genes/ total no. of genes
Information storage and processing			2/580
Transcription [K]			1/219
swp5124	0.429	FliA, Sigma-28 factor	COG1191
Replication, recombination, and repair [L]			1/182
swp0264	0.121	Transposase, putative	
Cellular processes and signaling			24/827
Defense mechanisms [V]			2/79
swp1329	2.036	AcrB, cation/multidrug efflux pump	COG0841
swp4709	2.024	EmrB, multidrug efflux system protein	COG0477
Signal transduction mechanisms [T]			2/159
swp0543	2.210	Rtn, diguanylate cyclase/phosphodiesterase	
swp4254	0.432	Putative diguanylate cyclase	
Cell wall/membrane/envelope biogenesis [M]			4/214
swp3209	2.548	OmpC, outer membrane protein (porin)	COG3203
swp4513	4.598	OmpC, outer membrane protein (porin)	
swp0383	0.331	MipA, MltA interacting	COG3713
swp5056	0.448	TagD, cytidyltransferase-like protein	COG0615
Cell motility [N]			16/134
swp5106	0.484	FlgC, flagellar basal body rod protein	COG1558
swp5107	0.365	FlgD, flagellar hook capping protein	COG1843
swp5108	0.398	FlgE, flagellar basal body rod protein	COG1749
swp5109	0.369	FlgF, flagellar basal body rod protein	COG4786
swp5110	0.403	FlgG, flagellar basal body rod protein	COG4786
swp5111	0.349	FlgH, flagellar L-ring protein	COG2063
swp5112	0.455	FlgI, flagellar P-ring protein	COG1706
swp5114	0.298	FlgK, flagellar hook-associated protein, putative	COG1256
swp5115	0.490	FlgL, flagellin, N terminus	COG1344
swp5117	0.321	FliB, flagellar biosynthesis protein, putative	
swp5118	0.252	LafA, flagellin, C terminus, flagellin, N terminus	COG1344
swp5122	0.489	FliK, flagellar hook-length control protein	COG3144
swp5125	0.267	MotA, MotA/TolQ/ExbB proton channel	COG1291
swp3912	0.426	Tar, methyl-accepting chemotaxis protein	COG0840
swp5082	0.436	FlhA, flagellar biosynthesis protein	COG1298
swp5086	0.484	FliP, flagellar transport protein	COG1338
Metabolism			5/1139
Amino acid transport and metabolism [E]			1/250
swp3956	2.224	GlnK, nitrogen regulatory protein PII	COG0347
Lipid transport and metabolism [I]			1/120
swp3551	2.003	PfaB, omega-3 polyunsaturated fatty acid synthase	
Inorganic ion transport and metabolism [P]			3/172
swp3955	2.301	AmtB, ammonia permease	COG0004
swp4022	0.424	BtuB, TonB-dependent receptor	COG1629
swp4055	0.489	CysC, adenylylsulfate kinase and related kinases	COG0529
Poorly characterized			7/575
General function prediction only [R]			3/298
swp0891	6.571	Azoreductase, putative	COG0431
swp0958	3.338	CBS domain protein	COG0517
swp3854	2.104	Sulfite oxidase and related enzymes	COG2041
Function unknown [S]			4/277
swp0326	2.043	Conserved hypothetical protein	COG2135
swp1406	2.215	Lipoprotein, putative	
swp4805	8.041	Predicted membrane protein	COG3776
swp4485	0.342	Conserved hypothetical protein	COG3422
No COG identified (hypothetical protein)			
swp0328	2.651	Conserved hypothetical protein	
swp2867	2.111	Conserved hypothetical protein	

(Continued on following page)

TABLE 3 (Continued)

Functional category and gene locus designation	Avg fold change (WP3ΔSW1/WP3)	Product and description	COG designation or no. of relevant genes/ total no. of genes
swp4118	4.528	Conserved hypothetical protein	
swp0260	0.429	Conserved domain protein	
swp0265	0.088	Hypothetical protein	
swp0384	0.246	Conserved hypothetical protein	
swp1931	0.387	Conserved hypothetical protein	
swp4362	0.248	Conserved hypothetical protein	
swp4486	0.454	Lipase family protein	
swp5102	0.448	Conserved hypothetical protein	
swp5103	0.497	Conserved hypothetical protein	

[a] COG, Clusters of Orthologous Groups.

teraction targets by comparing the gene transcription profiles of WP3 and WP3ΔSW1 at 4°C. Only 49 genes were found to be differentially expressed between these two strains (Table 3). To validate the microarray data, 11 genes, including those that were upregulated or downregulated or whose expression level was unchanged, were selected for quantitative real-time PCR (qPCR) (see Table S1 in the supplemental material). The same samples were used for the microarray and qPCR. The relative mRNA levels for each gene were calculated and log transformed. The correlation coefficient (R^2) between the data obtained by microarray and qPCR was 0.8695 (see Fig. S2 in the supplemental material), demonstrating that the microarray data were reliable and could be used for the follow-up analysis.

A total of 17 genes, 12 of which could be annotated with specific functions, were upregulated in the phage-free strain (Table 3). These functions included those related to membrane transport (*btuB*, *acrB*, *amtB*, *emrB*), secretion (*ompC*, porin), the two-component system (*glnK*), and lipid metabolism (*pfaB*). These results suggested that membrane function is important for the WP3 response to assembly and secretion of the filamentous phage. Upon deletion of the SW1 phage, 32 genes, 16 of which belonged to the lateral flagellum gene cluster (*flgCDEFGHIKL*, *flhA*, *fliABKP*, *lafA*, *motA*), were found to be downregulated (Table 3). In addition, 2 genes responsible for cell wall biogenesis (*mipA*) and purine metabolism (*cysC*) showed decreased expression in WP3ΔSW1. The majority of the genes which were differentially expressed in the phage-harboring and phage-free WP3 cells, such as those involved in lipid synthesis, membrane sensors, transporters, and secretors, were associated with the membrane, likely a consequence of the phage shock protein (Psp) response observed previously (36–38). The *psp* genes of WP3 was analyzed, an incomplete Psp system was found (swp3247 to swp3249 and swp3158 encoding *pspCBA* and *pspE*, respectively), and the transcriptional level of *pspCBA* was 2.5-fold higher in WP3 than in WP3ΔSW1 (see Fig. S3 in the supplemental material), indicating a lower activity of the Psp response when SW1 was absent.

Interestingly, a large portion of the genes for the lateral flagellum system were downregulated in WP3ΔSW1 at 4°C. Two representative genes from the lateral flagellar gene cluster, *lafA* and *fliA*, which encode lateral flagellin and flagellum-specific sigma-28 factor, respectively, were selected to confirm the microarray results. We measured the relative mRNA expression levels of *lafA* and *fliA* by qPCR, and in agreement with the microarray data, these two genes were downregulated 3.2- and 2.7-fold, respectively, compared to the WP3 strain results. The differential gene expression levels due to SW1 were detected only when the WP3 cells were cultivated at 4°C, and no differences in gene transcription were observed at 20°C (Fig. 2).

WP3 harbors two flagellum systems, the polar flagellar system for swimming motility and the lateral flagellar system for swarming motility. The two sets of flagellum systems were found to be inversely regulated by low temperature. The lateral flagellum sys-

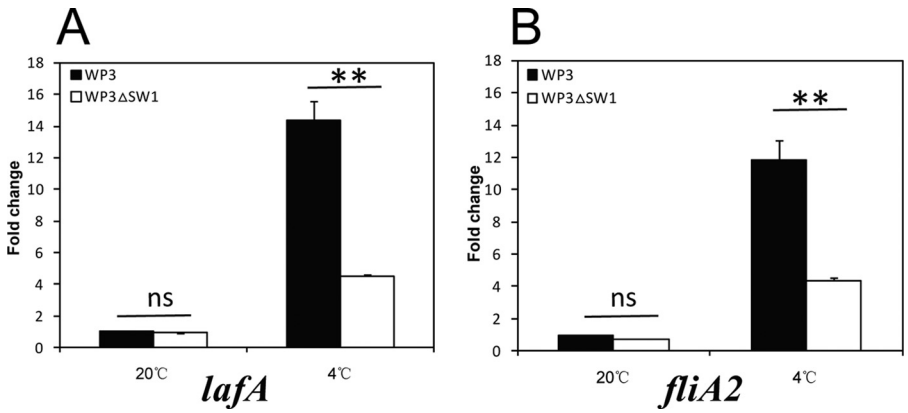

FIG 2 Assay of relative transcriptional levels of lateral flagellar genes *lafA* (A) and *fliA2* (B) in the SW1 mutant at different temperatures. The transcription level of WP3 at 0.1 MPa and 20°C was set as 1. The data shown represent the results of two independent experiments, and the error bars indicate the standard deviations. The data were analyzed by Student's *t* test. **, $P < 0.01$; ns, not significantly different.

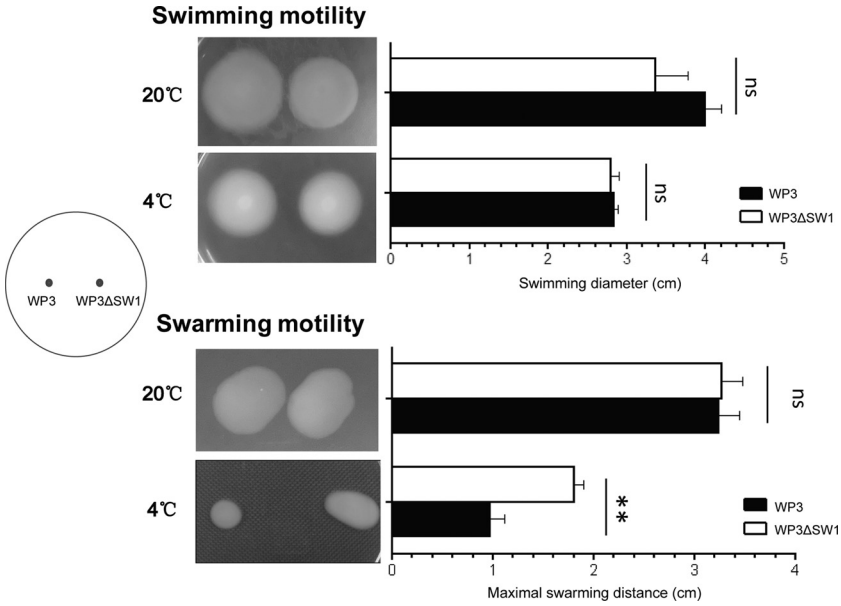

FIG 3 Swimming and swarming motility assays of the SW1 mutant at different temperatures. The data shown represent the results of at least three independent experiments, and the error bars indicate the standard deviations. The data were analyzed by Student's *t* test. **, $P < 0.01$; ns, not significantly different.

tem was found to be essential for motility and viability at low temperatures, as deletion of one key gene for lateral flagellum synthesis greatly decreased the growth of the cells at 4°C but had no influence at 20°C (39). The decreased expression of the lateral flagellum gene cluster was unexpected and raised the possibility that the SW1 phage impacts the cell swarming motility by controlling the expression of the lateral flagellum genes.

Deleting SW1 enhances the swarming motility of WP3 at 4°C. The motility of WP3 and WP3ΔSW1 was monitored at both 20°C and 4°C (Fig. 3). There was no significant difference between the swimming motility of WP3 and that of WP3ΔSW1 at either 20°C or 4°C. No differences in swarming motility were observed between WP3 and WP3ΔSW1 at 20°C, while at 4°C, the maximal swarming distance of WP3ΔSW1 was 186% longer than that of WP3, indicating that the swarming motility was significantly influenced by the deletion of SW1.

This result seems to be in contrast to the earlier observation that the transcription levels of lateral flagellar genes were downregulated in WP3ΔSW1 (Table 3 and Fig. 3). We suspect that these differences could be caused by the differential methods of cultivation in liquid and on solid surfaces, as swarming motility is not required in liquid medium. To confirm this, bacterial cells were sampled from the edge of a swarm colony, and the relative mRNA levels of the two lateral flagellar genes were quantified by qPCR (Fig. 4E). The expression level of the genes was significantly increased in WP3ΔSW1 (2.2- and 3.7-fold higher for *fliA2* and *lafA*, respectively) compared to WP3, in contrast to the results from liquid culture. Similarly, some membrane function-related genes (*acrB*, *ompC2*, *glnK*, *amtB*, and *emrB*) showed opposite transcription profiles under the two different culture conditions (see Fig. S4 in the supplemental material), suggesting that viscosity is an important factor influencing gene expression patterns. In addition, the TEM results showed that more lateral flagella were produced in WP3ΔSW1, and consistent with the genotype of these strains, abundant filamentous phages were produced by WP3, while there was no phage production by WP3ΔSW1 (Fig. 4C and D). There was no difference with respect to polar flagellum production between WP3 and WP3ΔSW1 in liquid culture (Fig. 4A and B). Taken together, our results clearly demonstrate that the SW1 phage has a significant impact on the expression of the lateral flagellar genes and the production of lateral flagella, thus influencing the swarming ability of the cells at low temperatures, as the surface density of the flagellum directly related to the swarmer cell motility in viscous environments (40). The underlying reason for the fact that SW1 has this influence only at 4°C is clear, given that SW1 is a cold-active phage which can propagate extensively and exert its influence only at low temperatures.

DISCUSSION

The impacts of viruses on the growth of their hosts have been investigated previously. For example, the excision of the CP4-57 prophage and the deletion of the cryptic prophages in *E. coli* decreased the host growth rates (15, 16). However, there were also reports indicating there was very little difference between phage-containing and phage-free bacteria. The growth rates of lysogenized strains (DMS3 phage) were similar to that of uninfected *Pseudomonas aeruginosa* PA14 in LB medium (35). Although the filamentous Pf4 phage is crucial for the biofilm life cycle of *Pseudomonas aeruginosa*, its deletion did not alter host bacterial growth (21). Little growth difference was observed between λ phage lysogen and nonlysogen in M9 minimal medium (supplemented with B12) using glucose as the sole carbon source. But striking growth differences (range, 20% to 30% per generation) were observed between the λ phage-infected and phage-free *E. coli* strains when cultivated in the same M9 minimal medium but using succinate as the sole carbon source (14). These results indicated that the impacts of phage on the growth of its host are rather complicated, depending largely on the cultivation conditions of the strains. In our study, no growth difference between WP3 and the phage-free WP3ΔSW1 strain was observed under our detect-

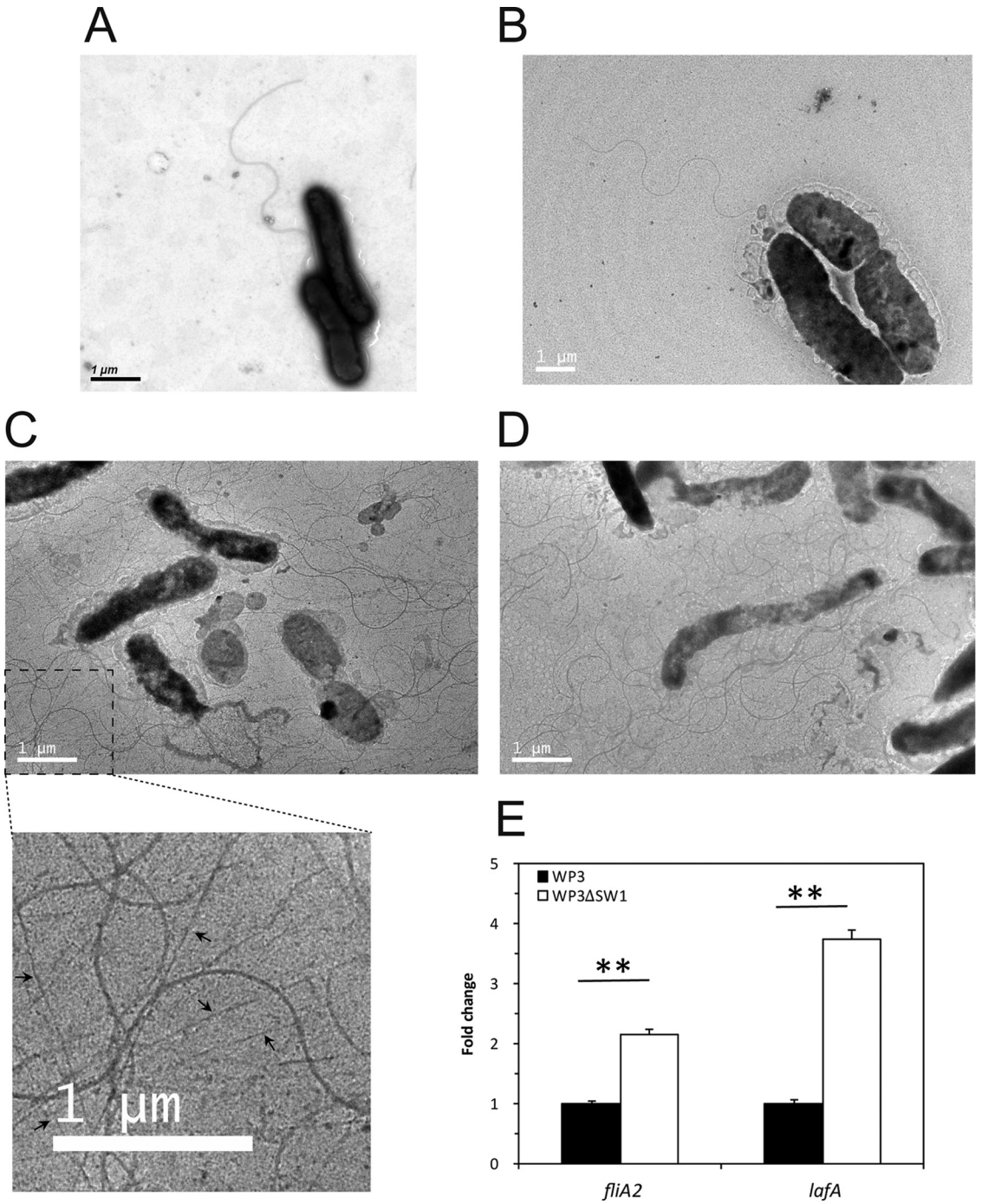

FIG 4 (A to D) Transmission electron microscopic observation of WP3 (A and C) and WP3ΔSW1 (B and D) swimming cells (A and B) and swarming cells (C and D) cultured at 4°C. (E) Assay of relative transcription levels of lateral flagellar genes. The transcription level of WP3 was set as 1. The data shown represent the results of two independent experiments, and the error bars indicate the standard deviations. The data were analyzed by Student's *t* test. **, $P < 0.01$. Arrows indicate the presence of a filamentous phage.

ing conditions (see Fig. S1 in the supplemental material). As the range of cultivation conditions we checked was rather limited, further studies are necessary to check and compare the growth rates of the cells under additional conditions such as anaerobic cultivation using different electron acceptors (lactate, nitrate, etc.) to see if there are any growth differences between the phage-infected and phage-free strains.

The influence of phages on the motility of cells has been documented previously. For example, it was reported that the swimming motility of *E. coli* was induced after deletion of the prophage CP4-57 (16), while an Stx2 phage-infected strain showed enhanced swimming motility compared to that seen with the uninfected bacterial host (13). Here, we showed that phage SW1 has no influence on the swimming motility of the host cells, while the

deletion of SW1 does cause a significant enhancement of the swarming motility of the cells at 4°C (Fig. 3). It is still unclear how SW1 influences the lateral flagellar system of the host cell. A previous study of *P. aeruginosa* indicated that the DMS3 bacteriophage inhibits the swarming motility of the host by interacting with the CRISPR (clustered regularly interspaced short palindromic repeats) region (35). We analyzed the CRISPR sequence in the WP3 genome and failed to find any sequence homologous to that of SW1 in the spacer region (data not shown). In contrast to DMS3, SW1 integrates with the WP3 genome at specific sites (*attB*), and the adjacent genes are not relevant to lateral flagellar genes (39). Therefore, SW1 influences the swarming motility of WP3 by another mechanism which is dependent on temperature. It has been reported that bacteriophage-encoded regulators bind to some host genes and repress or activate transcription (14, 41, 42). SW1 harbors a putative repressor, FpsR, which possesses a Cro/CI-type helix-turn-helix DNA binding motif (22). Our preliminary study showed that FpsR is not involved in low temperature-inducible phage production and that other, unknown proteins or mechanisms are utilized by this cold-active phage (unpublished data). The molecular mechanism will need to be elucidated in future studies.

Motility plays an important role in the adaptation of bacteria to different environments, and flagella are the key organelles for bacterial locomotion, helping them move toward favorable conditions or avoid detrimental environments (43, 44). Motility using flagella is very expensive for the cell, in terms of the large amount of genes and energy required for flagellar biosynthesis and functioning (45), and it was estimated that these activities account for approximately 2% of the biosynthesis energy expenditure of *E. coli* (46). The swarming motility of WP3 at 4°C has been shown to be much lower than that at 20°C (Fig. 3), while the activity and the production of SW1 are activated primarily at low temperatures (22, 25). These results suggest that WP3 must balance its maintenance of SW1 with its need for swarming activity. The concept of the biological trade-off, by which an organism receives an advantage by performing one function while simultaneously facing a disadvantage for performing another function, is central to most theories about the diversity of life on Earth (47). Such trade-offs are likely due to the limitations of cellular resources and physical or chemical constraints (48). At low temperatures, both the affinity for substrate uptake and the biochemical reaction rates decrease (49–51). As the production of both the phage and the lateral flagella is energy consuming, it is important for the bacterium to maintain a balance to maintain viability in the limited-energy cold environment. Such a trade-off between phage production and swarming motility would be an important adaptive mechanism of WP3 for survival and growth at low temperatures. From this aspect, it would be interesting to investigate the effect of different substrates on motility and illustrate the relationships among phage production, host metabolism, and motility.

The SW1 deep-sea filamentous phage has demonstrated the ability to regulate the expression of a variety of host genes, most impressively the lateral flagellar genes, and is thus able to reduce the host cell's swarming motility. WP3 was originally isolated from deep-sea sediment, where swarming is most likely the dominant motility pattern of the cells; such an interaction between SW1 and WP3 would confer fitness to the cells and be adaptive for cold deep-sea environments.

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