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Potential Swimming Motility Variation by AcrR in *Escherichia coli* S

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AcrR, the toxic-compounds-response regulator, regulates motility in microorganisms, presumably to escape from toxic environments. In this study, the genome-wide target genes of AcrR were investigated in a Δ*acrR* mutant strain by microarray analysis. In the absence of AcrR, the transcription of most flagella/motility genes was highly increased. In addition, flagella formation was increased in this mutant strain. Motility assays revealed that AcrR modulates swimming motility, but not swarming.

Keywords: AcrR, flagella, motility, swimming, swarming

The AcrR protein of *Escherichia coli* is a local transcriptional repressor of AcrAB-TolC, the main multidrug efflux system of *E. coli* with broad substrate specificity [13]. The *acrR* gene is divergently located 141 bp upstream of the *acrAB* operon [11, 15]. The presence of toxic compounds induces the transcription of *acrAB*, which is primarily mediated by global regulators MarA and SoxS, and AcrR that plays the role of a specific repressor modulating the level of transcription [6, 11, 17]. AcrR is a TetR-type repressor composed of an N-terminal domain containing a DNA-binding HTH motif, and a C-terminal domain containing a unique ligand-binding sequence [8, 15, 18]. The binding of toxic compounds to the C-terminal domain of AcrR triggers a conformational change in its N-terminal domain, and AcrR is released from DNA, allowing the transcription of the target gene [3, 18].

A previous study showed that AcrR represses the transcription of genes of the local target *acrRAB* and of the global regulators *marRAB* and *soxRS* by directly binding to their promoter region [6]. In addition, the Δ*acrR* strain of *E. coli* is hypersensitive to solvents, but overexpression of *acrR* (*acrR*$^+$ strain) improved the tolerance to solvents [6], implying that AcrR is more involved in the regulation network than was previously identified. Here, we investigated the genome-wide targets for AcrR by using microarrays and found that it regulates the transcription of most genes encoding flagella/motility. The phenotypic changes of the Δ*acrR* strain were studied using motility assays.

Genome-wide transcription changes in the absence of AcrR were investigated by performing DNA microarray of *E. coli* strains BW25113 (wild-type) and JW0453 (Δ*acrR*). Total mRNA was isolated from *E. coli* cells at OD_{600} = 0.8 using the RNAProtect Bacterial Reagent/RNeasy minikit/RNase-free DNase (Qiagen, Germany), and transcribed to cDNA with SuperScript II reverse transcriptase (Invitrogen, USA). The cDNA was fragmented (using DNase I; Pierce), labeled by biotin (GeneChip IVT labeling kit; Affymetrix, USA), hybridized to a microarray chip (GeneChip *E. coli* Genome 2.0 Array; Affymetrix, USA), washed, and stained (GeneChip hybridization, wash and stain kit; Affymetrix, USA). The array was analyzed using the GeneChip Scanner

3000 7G (Affymerix, USA). Data were analyzed using Robust Multi-array Average, and the trimmed mean target intensity of each array was arbitrarily set to 100. Tests were duplicated independently.

Gene expression of the Δ*acrR* strain was compared with that of the wild type. Differences of over 2.0-fold were considered statistically significant (Fig. S1−S4, Table S1−S3), and the microarray data were deposited in the Gene Expression Omnibus repository with the accession number GSE84847.

The genes with increased transcription of over 2.5-fold were summarized to prevent a false-positive value, and the corresponding genes were considered to be regulated by AcrR. In the Δ*acrR* strain, 184 genes showed increased expression, and these were categorized into seven functional groups: carbon utilization (43), chaperone (12), flagella/motility (44), genetic information (9), regulation (14), transport (23), and others (23). The transcription of most flagella/motility genes (44/50 genes) was significantly increased in the Δ*acrR* strain (Table 1), indicating that AcrR affects the bacterium's motility. The fold change of flagella/motility genes in each microarray is shown in Table S4. The flagellar regulons are divided into three classes (I, II, and III) by hierarchical transcription [9, 10]. The expression of the master regulators FlhD and FlhC (class I) [1] were increased by 2.3- and 2.6-fold in the Δ*acrR* strain, respectively, which directly induced the expression of the class II genes *flgA*, *flgBCDEFGHIJ*, *flhBAE*, *fliAZY*, *fliE*, *fliFGHIJK*, and *fliLMNOPQR* (Table 1). Class II genes encode structural components of flagella and two important regulation factors, FilA and FilZ. FilZ, an anti-σ^s factor that inhibits curli fimbriae-mediated adhesion and biofilm formation [14], was increased 6.7-fold in the Δ*acrR* strain. FilA, a specific sigma factor σ^{28}, is required for the transcription of class III genes [9, 10]. Genes for chemotaxis, *tar-tap-cheRBYZ* and *motAB-cheAW*, and flagellin, *fliC*, are under the control of FliA (class IIIa), and genes *flgMN*, *flgKL*, and *fliDST* are under the control of both FlhD/FlhC and FliA (class IIIb) [9, 10]. FilA was induced 7.4-fold in the Δ*acrR* strain. The expression of class III genes in the Δ*acrR* strain was increased (Table 1), indicating that AcrR participates in flagella formation/motility by modulating FlhD/FlhC or FliA. In addition, Ruiz and Levy [17] reported that 29 genes for flagella/motility were highly induced in the Δ*acrB* strain, which belong to classes II and IIIa. The deletion of *acrB* inactivated the AcrAB-TolC pump and caused the accumulation of toxic cellular compounds [17], which serves as an effector for AcrR. It supports our finding that the flagellar motility is regulated by AcrR.

The gene region of *flhDC* has potential AcrR-binding sites, of which -36~-27 (from transcription start) is the possible site to repress transcription (Fig. 1). The 5′-gtTGTATGTg-3′ sequence at -36~-27 shows 70% similarity to the second half of the AcrR-binding motif (5′-TACATACATT-tatg-AATGTATGTA-3′) [16], on which AcrR could bind loosely and allow for quick escape in response to toxic compounds.

```
+559 tcactcatga tcaggccctt ttcttgcgca gcgcttcttc aggctgatta acatcattca
       ←flhC
+499 gcaagcgtgt tgagagcatg atgccggtat gaatttgctg gagatcgtca acgcgggaat
                                      flhC +1       -10
+439 cttgcgtcaa ctgagtaatc gtctggtggc tgtcaaaacg gaagtgacaa accagttgat
        -35
+379 tggtttctgc cagcttaacc atttgcggaa gagtcagtgc cgctaacgtt gtcgccattt

+319 cttcatttat gccgagacga aacatagcgg acgctttgtc ctgaacaatc aaacgctgtg
                                        AcrR site
+259 caagtagtaa atatgacaag ttgatgtcat aAATGTgTtT cagcaactcg gaggtatgca

+199 ttattcccac ccagaataac caactttatt tttatgcggt ttcaccgcac cccgtgatgt
     ←flhD      SD
+139 cgccgggaag ccccggtaaa aaataattag cattagaata gttgcgataa gctgcaataa

 +79 gcagaaccac ctttttggtt taatatgtcc ttacaaatag aaatgggtct ttacacttat
                                                        AcrR site
 +19 ctaagatttt tcctaaatcg acgcaactgt actcgtcact acacgcACAT ACAacggagg
                   flhD +1     -10                       -35   AcrR site
 -42 ggggctgcga ttttcaataa tgcgtgatgc agatcacaca aaacactcaa ttacttaACA

-102 TAaATgggta atgactccaa cttattgata gtgttttatg ttcagataat gcccgatgac
```

Fig. 1. Potential AcrR-binding sites in the promoter regions of the *flhD* and *flhC* genes (shaded) [16].

The transcription-start site of the *flhDC* operon is indicated as a bent arrow with +1. The Shine-Dalgarno (SD) sequence and translation initiation codon are in bold. The promoter regions (-10 and -35) are underlined.

Table 1. The relative transcription of flagellar/motility genes in *acrR* deletion strain (Δ*acrR*) compared with wild-type by DNA microarray.

b No.	Flagella class[a]	Gene[b]	Fold change[c]	*p*-Value[d]	Description
Flagellar gene cluster 1					
b1070	IIIa	*flgN*	**3.9**	**0.001**	Substrate-specific chaperones of the flagellar export system
b1071	IIIa	*flgM**	**3.5**	**0.012**	Anti-sigma factor σ^{28}
b1072	II	*flgA*	**5.9**	**0.011**	Assembly protein for flagellar basal-body periplasmic P ring
b1073	II	*flgB*	**8.6**	**0.008**	Flagellar component of cell-proximal portion of basal-body rod
b1074	II	*flgC*	**7.4**	**0.005**	Flagellar component of cell-proximal portion of basal-body rod
b1075	II	*flgD*	**6.5**	**0.017**	Flagellar hook assembly and basal body rod modification protein
b1076	II	*flgE*	**7.7**	**0.001**	Flagellar hook protein
b1077	II	*flgF*	**4.5**	**0.033**	Flagellar component of cell-proximal portion of basal-body rod
b1078	II	*flgG*	**7.1**	**0.019**	Flagellar basal-body rod protein that compose motor complex
b1079	II	*flgH*	**7.9**	**0.017**	Basic subunit protein of LPS ring of the flagellar basal body
b1080	II	*flgI*	**2.6**	0.179	Flagellar P-ring protein
b1081	II	*flgJ*	**4.8**	**0.046**	Flagellar rod assembly protein and murein hydrolase
b1082	IIIa	*flgK*	**5.5**	**0.071**	Flagellar hook-filament junction protein
b1083	IIIa	*flgL*	**4.5**	0.088	Flagellar hook-filament junction protein
Flagellar gene cluster 2					
b1878	II	*flhE*	1.5	0.102	Operon with flhBA which encode type 3 flagellar export proteins
b1879	II	*flhA*	**3.1**	**0.026**	Flagellar export pore protein
b1880	II	*flhB*	**3.0**	**0.051**	Flagellin export apparatus
b1881	IIIb	*cheZ*	1.7	0.174	Chemotaxis regulator and protein phosphatase for CheY
b1882	IIIb	*cheY*	**2.1**	0.376	Chemotaxis regulator transmitting signal to flagellar motor
b1883	IIIb	*cheB*	1.7	0.539	Chemotaxis response regulator protein-glutamate methylesterase
b1884	IIIb	*cheR*	**2.1**	0.215	Chemotaxis protein methyltransferase
b1885	IIIb	*tap*	**4.3**	0.337	Methyl-accepting chemotaxis protein IV, peptide sensor receptor
b1886	IIIb	*tar*	**13.4**	0.379	Methyl-accepting chemotaxis protein II
b1887	IIIb	*cheW*	**3.2**	0.356	Chemotaxis signal transducer
b1888	IIIb	*cheA*	**4.7**	0.155	Sensory histidine kinase in chemotaxis signal transduction system
b1889	IIIb	*motB*	**4.0**	0.215	Protein that enables flagellar rotation and linking torque machinery
b1890	IIIb	*motA*	**7.2**	0.134	Proton driven stator protein of flagellar rotation
b1891	I	*flhC**	**2.6**	**0.052**	Component of FlhDC transcriptional regulator of flagellar class II
b1892	I	*flhD**	**2.3**	**0.049**	Component of FlhDC transcriptional regulator of flagellar class II
Flagellar gene cluster 3					
b1920	II	*fliY*	1.2	0.908	Member of extracellular bacterial solute-binding protein family III
b1921	II	*fliZ**	**6.7**	**0.021**	Transcriptional repressor of genes involved in curli expression
b1922	II	*fliA**	**7.4**	**0.014**	σ^{28} for initiation of transcription of genes in motility and flagellar
b1923	IIIb	*fliC*	**9.4**	0.411	Flagellar filament structural protein flagellin
b1924	IIIa	*fliD*	**5.6**	**0.055**	Flagellar filament capping protein
b1925	IIIa	*fliS*	**7.5**	0.066	Substrate-specific chaperones of the flagellar export system
b1926	IIIa	*fliT*	**3.4**	**0.042**	Substrate-specific chaperones of the flagellar export system
Flagellar gene cluster 4					
b1937	II	*fliE*	**5.0**	0.102	Flagellar basal-body component
b1938	II	*fliF*	**5.6**	0.086	Flagellar basal-body supermembrane-ring and collar protein
b1939	II	*fliG*	**6.2**	**0.015**	Flagellar motor switching and energizing component

Table 1. Continued.

b No.	Flagella class[a]	Gene[b]	Fold change[c]	p-Value[d]	Description
Flagellar gene cluster 4					
b1940	II	*fliH*	**4.8**	**0.017**	Negative regulator of FliI ATPase activity
b1941	II	*fliI*	**5.4**	**0.019**	Flagellum-specific ATP synthase
b1942	II	*fliJ*	**4.9**	**0.009**	Flagellar protein
b1943	II	*fliK*	**5.0**	**0.010**	Flagellar hook filament junction that controls hook length
b1944	II	*fliL*	**7.8**	**0.023**	Flagellar biosynthesis protein that affects rotational direction
b1945	II	*fliM*	**4.3**	**0.072**	Flagellar motor switch protein
b1946	II	*fliN*	**7.4**	**0.027**	Flagellar motor switching and energizing component
b1947	II	*fliO*	**5.7**	**0.029**	Flagellin export apparatus, integral membrane protein
b1948	II	*fliP*	**4.2**	**0.054**	Flagellin export apparatus, integral membrane protein
b1949	II	*fliQ*	**3.4**	**0.034**	Flagellin export apparatus, integral membrane protein
b1950	II	*fliR*	**2.9**	0.128	Flagellin export apparatus, integral membrane protein

*The full microarrary result is listed on the Gene Expression Omnibus database with the accession number GSE84847 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE84847) and in Table S1.

[a]Classification of flagella genes according to Liu and Matsumura [9].

[b]The asterisk (*) indicates the genes related to transcriptional regulation.

[c]Fold change shows the average value of the independent microarrays. Fold change over 2.0 is indicated in bold.

[d]p-Value under 0.005 is indicated in bold.

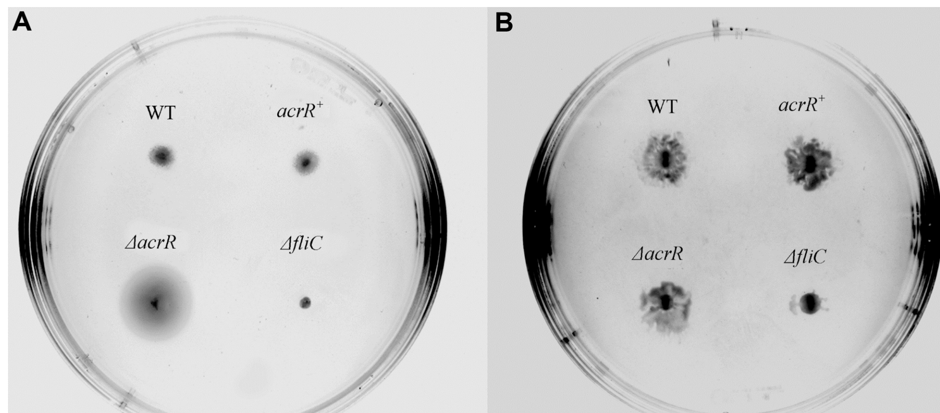

Fig. 2. Swimming (**A**) and swarming (**B**) motility assays.
The plates were photographed under white light.

Local repressors such as EmrR or AcrS for multidrug resistance can also regulate distally located genes [4, 12]. The promoter region for the *fliAZ* gene has no possible binding motif for AcrR (data not shown). Therefore, the flagella/motility regulation by AcrR could be mediated by FlhDC. Further research should focus on the direct binding of AcrR to the *flhDC* promoter.

The effect of AcrR on the motility of the *E. coli* strains BW25113 (wild-type), BW25113 containing pNTR-SD::*acrR* (*acrR*+), JW0453 (*ΔacrR*), and JW1908 (*ΔfliC*) was tested using swimming and swarming assays [5]. For the swimming assay, one colony was inoculated deeply into a fresh LB agar plate (0.3% agar and 200 μM IPTG) and incubated at 33°C for 12 h. For the swarming assay, one colony was inoculated onto the surface of a 30-min-dried LB agar plate (0.6% agar, 0.5% glucose (w/v), 200 μM IPTG) and incubated at 37°C for 38 h. The Δ*fliC* mutant was used as the negative control for both assays [5], because *fliC* encodes flagellin, the primary structural subunit of flagellum [7]. Swimming was greatly increased in the Δ*acrR* strain compared with that in the wild-type and *acrR*+ strains (Fig. 2A), whereas swarming was similar among the three strains (Fig. 2B).

Therefore, AcrR negatively regulates swimming but not swarming. The same phenomena were also observed in the Δ*acrB* strain [17]. However, it is unclear why only swimming motility is influenced by AcrR. Two recent studies about genome-wide motility showed that most flagella genes affect both types of motility (Table S5) [2, 5]. Certain genes—*flgL*, *flgN*, *fliD*, *fliS*, *tsr*, and *cheB*—are required for only swimming [2]; *fliR* is required only for swarming [5] (Table S5). However, it does not provide information on the the differentiated mediation of AcrR to regulate swimming motility.

In addition, the increase in flagella formation in the Δ*acrR* strain compared with that in the wild-type and $acrR^+$ strains was observed by using energy-filtered transmission electron microscopy (Fig. S5). Although the reason why swimming motility, but not swarming, increased with the flagella formation is not yet known, the regulation of flagella formation by AcrR is confirmed.

In conclusion, the deletion of *acrR* in *E. coli* induced transcription of most flagella/motility genes and increased flagella formation. This implies that the toxic-compounds-response regulator AcrR participates in the regulation of motility to escape toxic compounds, which should be mediated by the master regulator of flagella/motility genes, presumably FlhDC. AcrR modulates swimming motility, but not swarming motility.

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