TABLE 1. Data collection statistics

Parameter	Value
Wavelength (Å)	0.979277
Resolution (Å)	87.7–3.4 (3.6–3.4)[a]
Highest-resolution shell (Å)	3.52–3.4
No. of observed reflections	80,998
No. of unique reflections	7,246
Completeness (%)	95.9 (93.4)
R_{sym} (%)[b]	7.0 (28.9)
Avg $I/\sigma(I)$	7.3 (1.8)
Mosaicity (°)	0.351

[a] The values in parentheses are data for the highest-resolution shell.
[b] $R_{sym} = 100(\Sigma|I - \langle I\rangle|/\Sigma I)$, where I is the intensity of the individual measurement and $\langle I\rangle$ is the average intensity from multiple observations.

TABLE 2. Refinement statistics

Parameter	Value
Resolution range (Å)	87.7-3.4 (3.6–3.4)[a]
No. of reflections in working set	6,906
No. of reflections in free-*R* set	340
No. of protein atoms[b]	1,352
R factor (%)[c]	22.4 (33.7)
Free-*R* factor (%)[d]	28.6 (41.7)
RMSD (bond lengths) (Å)	0.014
RMSD (bond angles) (°)	1.76
% of ϕ and ψ angles[e]	
Most favored	77.3
Additional allowed	20.0
Generously allowed	1.3
Disallowed	1.3

[a] The values in parentheses are data for the highest resolution shell.
[b] Nonhydrogen atoms only.
[c] R factor = $100[\Sigma(||F_{obs}| - |F_{calc}||)/\Sigma|F_{obs}|]$.
[d] R_{free} is the R factor for a selected subset of reflections (5%) that were not included in the refinement calculations.
[e] Stereochemistry was assessed with PROCHECK (38).

incorporating a distribution of sedimentation coefficients by using the software package DCDT (60).

FliN/FliM ratio in purified complex. The purified FliM-FliN complex was dissociated in SDS-PAGE loading buffer, and the subunits were resolved on 15% polyacrylamide gels. The gels were stained with Coomassie blue G-250, and bands were quantified by using video densitometry and the program NIH-IMAGE. Proteins were loaded at a range of concentrations, and plots of absorbance versus loading were used to estimate relative protein levels. As a further check, purified FliM and FliN were resolved on SDS—12% PAGE gels and visualized by silver staining by using a protocol that reportedly has been optimized for uniformity and linearity (49, 80). Bands were quantified by video densitometry by using the same method that was used for Coomassie blue-stained gels, except that a green filter was used.

Crystallization and data collection. Following sizing chromatography, FliN was concentrated to 3 mg/ml. Crystals were grown at room temperature in sitting drop trays with a well solution containing 18% (vol/vol) 2-methyl-2,4-pentanediol and 100 mM morpholineethanesulfonic acid (MES) (pH 5.9). The drops were set up with a ratio of well solution to protein of 1:1. The crystals grew to full size in about 12 h. The crystals used for data collection typically were 200 by 50 by 50 μm.

FliN crystals were cooled by rapid immersion in liquid nitrogen after a brief (ca. 10-s) soak in 25% 2-methyl-2,4-pentanediol–100 mM MES (pH 5.9). X-ray diffraction data were collected at 100K by using a charge-coupled device Quantum 4 detector (ADSC) at ALS beamline 5.0.2. Data were processed (Table 1) by using DENZO and SCALEPACK (55). The crystals belonged to space group $P3_121$ with the following unit cell dimensions: $a = 100.2$ Å and $c = 87.9$ Å. The asymmetric unit contained two FliN molecules, and the solvent content was approximately 70%.

Structure determination and refinement. The crystal structure of a similar but slightly smaller *T. maritima* FliN construct was recently solved by the Joint Center for Structural Genomics (PDB accession code 1O6A). We were able to obtain phases for our data by performing molecular replacement using the programs AMORE (52) and MOLREP (73) and the coordinates for 1O6A. The resulting maps were clear and showed continuous density characteristic of α-helices and β sheets throughout the model region. The program O (29) was used for model building, and refinement calculations were performed with REFMAC5 (50). Refinement statistics are shown in Table 2. Figures were prepared by using the programs PYMOL (12) and RasMol (57).

Hydrophobic-patch mutant. Residue Val113 in the hydrophobic patch of FliN was mutationally replaced with Asp by using the Altered Sites (Promega) procedure with *fliN* cloned in plasmid pLS4 (40). The mutation was confirmed by DNA sequencing. The mutated gene was then transferred into plasmid pHT39, which allowed controlled expression of FliN from the *lac* promoter (65). Assays for swarming in soft agar, swimming in liquid, and flagellation were performed as described previously (66) by using the *fliN* deletion strain DFB223 transformed with wild-type or mutant plasmids. The swarm plates contained tryptone broth and 0.28% agar. Flagella were stained by a wet mount procedure (23).

Protein structure accession number. Coordinates and diffraction data for the FliN crystal structure have been deposited in the RCSB Protein Data Bank under accession number 1YAB.

RESULTS

FliN from *T. maritima* functions in *E. coli*. For the structural study and certain other experiments below we used the FliN protein from *T. maritima*, whereas previous information on FliN function and localization has come mainly from studies of *Salmonella enterica* serovar Typhimurium (19, 27, 81) or *E. coli* (65, 67). *T. maritima* is a hyperthermophile with a single polar flagellum (25). The amino acid sequences of *T. maritima* and *E. coli* FliN are 39% identical overall and 55% identical in the segment that is most important for function and whose structure is described below. To further validate the use of the *T. maritima* protein, we used a soft-agar swarming assay to test the ability of the *T. maritima fliN* gene to complement an *E. coli fliN*-null strain. *fliN*-null cells are nonflagellate, immotile, and unable to swarm in soft agar (65). When transformed with a plasmid expressing the *T. maritima fliN* construct, the cells swarmed at a rate that was about 20% of the wild-type rate (Fig. 2). When examined under the microscope, cells expressing *T. maritima* FliN showed fair motility but were more tumbly than wild-type cells. This functional complementation indicates that *T. maritima* FliN provides a valid structural model for the protein from *E. coli*.

Crystal structure of FliN. A construct encompassing residues 23 to 154 of *T. maritima* FliN was overexpressed in *E. coli* and purified, and it formed crystals that diffracted to a resolution of about 4 Å. Selenomethionine-substituted protein was

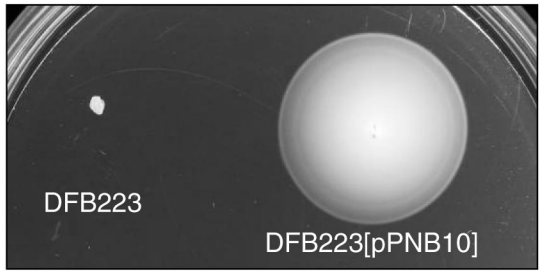

FIG. 2. Complementation of the *fliN*-null strain DFB223 by the *fliN* gene from *T. maritima*. This strain has an in-frame chromosomal deletion of *fliN* (65). Plasmid pPNB10 encodes residues 23 to 154 of the *T. maritima* FliN protein. A tryptone plate containing 0.28% Bacto agar (Difco) was spotted with 1 μl of an overnight culture of each strain and incubated at 32°C for 24 h.