

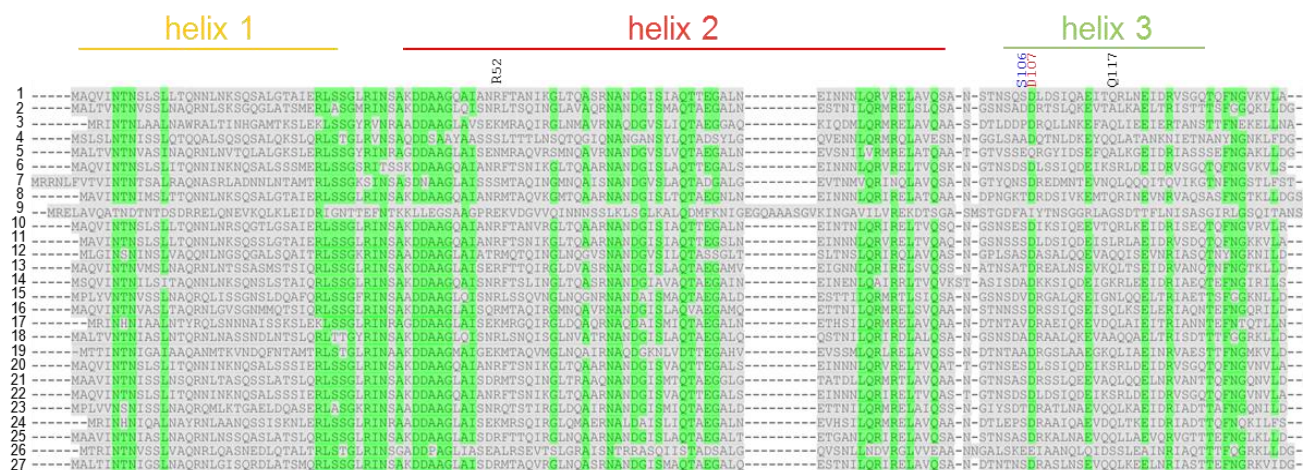
SJW1655 A449V		N430D 2 R431C 2 R431S 3 R431H 4 F432C 2 A449V 11	HFG195 A80V		V125L 2 S126F 1 Q128H 1 Q128L 1 T129A 1 T129F 1 T142P 2 Q146L 4 G148S 2 D412A 2 A416T 1 V418F 1 R422C 1 R422G 1 A427E 1 A427T 1 A427V 3 R431C 4 S434F 1 A435D 1 N438H 1 N438K 1 G440D 3 A449S 1 A449T 1 R450H 2 S451I 1 S463F 1 N464K 1 Q481L 1 N483H 1
* V449A 32 * V449G 10 D107E 4 G426A 12 G426S 35 S448F 1	HFG180 G70C		*V80A 3 *V80M 4 N7D 1 L11Q 1 Q14L 1 N16K 1 S39G 1 A45S 1 Q47K 1 A55T 1 A55V 1 N68K 1 Q75R 1 T76S 2 T77A 1 E78G 1 N82S 4 N86K 1 N87S 2 V96A 1 V96L 1 Q97H 1 N103K 1 Q105P 1 S106F 1 T116A 1 T116I 1 R118C 2 R118L 1 N120K 1 D123E 1		
SJW1660 G426A		*C70G 11 *C70S 21 Q62L 3 N82H 1 T102N 1 N103K 1 N103S 1 S106F 1 S106Y 1 R118C 3 Q128H 1 I145V 2 Q146L 4 A414V 1 A416V 4 R422C 3 R422H 1 A427S 1 A427T 2 A427V 1 R431C 9 F432L 1 F432S 2	*A426G 5 A40G 2 Q47K 1 N51S 3 N87D 2 N87H 1 N87K 3 Q97H 3 Q97L 6 S106Y 2 Q117P 6 E121D 3 V125E 1 V125L 5 D151Y 1 I155S 1 L408P 2 A416V 13 S423T 2 A427G 1 A427T 10 A427V 9		

Figure S1 The second mutations and their numbers isolated.

In the gray cells, strain names (upper line) and their first mutation sites (lower line) are described.

The all second mutations (left column) of the all revertants isolated from each parent strain and the numbers of the revertants isolated (right column) are summarized. The second mutations denoted by an asterisk are not contained in the second mutation sites because the sites are the first mutation site.

A



B



Figure S2 Multiple sequence alignment of FliC

Twenty seven FliC sequences of N-terminal (A) and C-terminal (B) regions are aligned (see Material and Methods). The residues shaded with green and red boxes indicate that the residues are conserved among more than 22 bacteria (i.e. >80%) and in all bacteria (i.e. 100%), respectively. The bars with different colors at the upper side show the helix 1 to 5 in *Salmonella* flagellin. The numbers (1 to 27) at the left side and the right side in panels A and B, respectively, indicate the bacterial names as follows: 1, *S. typhimurium* LT2; 2, *Pseudoalteromonas haloplanktis* TAC125; 3, *Symbiobacterium thermophilum* IAM 14863; 4, *Ralstonia solanacearum* GMI1000; 5, *Geobacter*

sulfurreducens PCA; 6, *Erwinia tasmaniensis* Et1/99; 7, *Zymomonas mobilis* ZM4; 8, *Erwinia carotovora* SCRI1043; 9, *Heliobacterium modesticaldum* Ice1; 10, *Photorhabdus luminescens* TTO1; 11, *Yersinia pestis* CO92; 12, *Burkholderia pseudomallei* K96243; 13, *Xanthomonas axonopodis* 306; 14, *Sodalis glossinidius morsitans*; 15, *Idiomarina loihiensis* L2TR; 16, *Legionella pneumophila* Philadelphia 1; 17, *Pelotomaculum thermopropionicum* SI; 18, *Pseudomonas aeruginosa* PAO1; 19, *Rhodobacter sphaeroides* 2.4.1; 20, *Escherichia coli* K-12 MG1655; 21, *Janthinobacterium* sp. Marseille; 22, *Shigella flexneri* 301; 23, *Saccharophagus degradans* 2-40; 24, *Geobacillus kaustophilus* HTA426; 25, *Thiobacillus denitrificans*; 26, *Rhodopirellula baltica* 1; 27, *Desulfotalea psychrophila* LSv54.

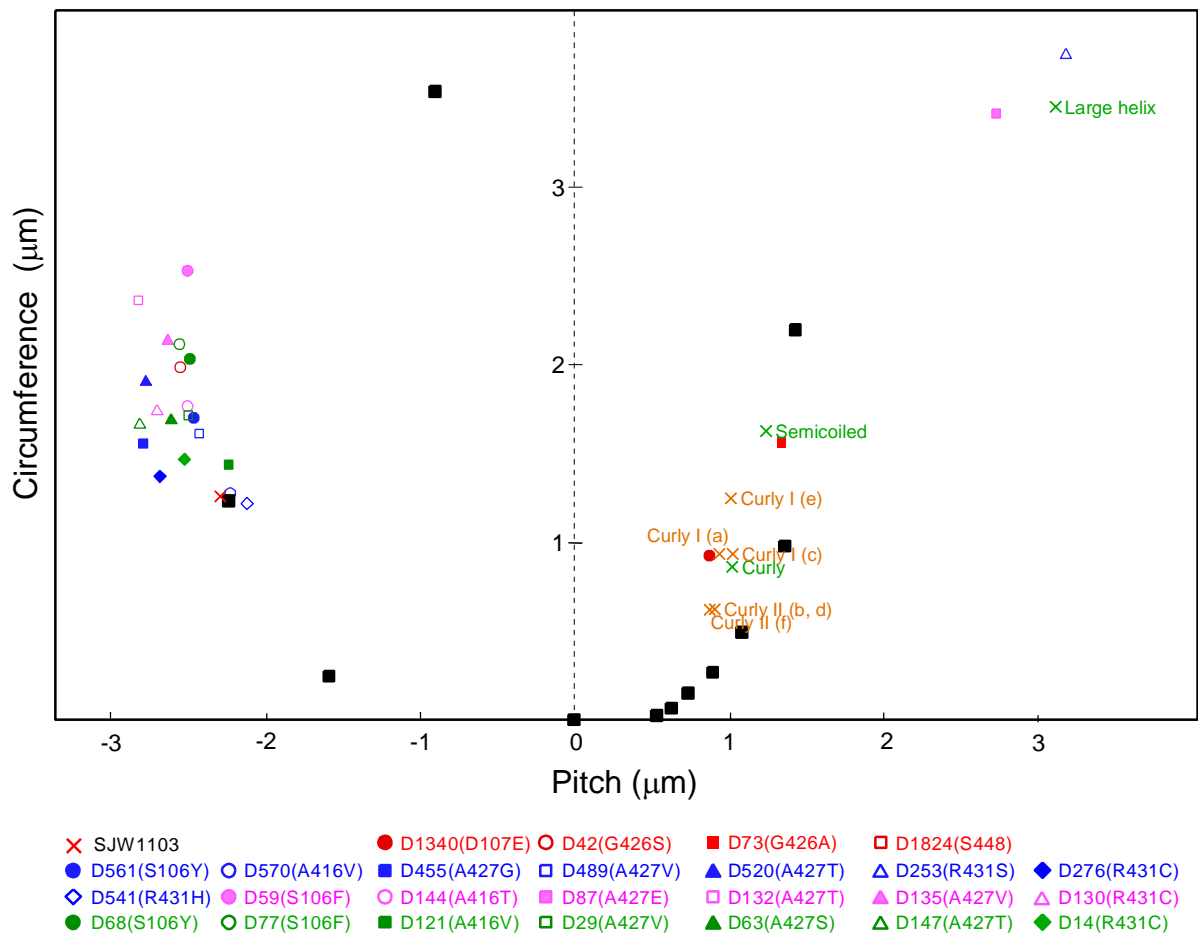


Figure S3 Pitch vs circumference graph of the static filament shapes in Fig. 5B

The helical parameters of the static filaments shapes of WT and the 25 revertants are plotted on Pitch vs circumference graph (Shibata *et al.*, 2005). The plotted positions of the symbols were averages of the calculation values from 5 independent filaments of each strain. Standard deviations of X and Y of WT were 0.10 and 0.09 and those are represented as WT (0.10, 0.09). Similarly, D1340 (0.07, 0.03), D42 (0.14, 0.17), D73 (0.08, 0.19), D561 (0.16, 0.15), D570 (0.10, 0.15), D455 (0.15, 0.10), D489 (0.08, 0.12), D520 (0.13, 0.10), D253 (0.10, 0.24), D276 (0.14, 0.19), D541 (0.14, 0.08), D59 (0.07, 0.13), D144 (0.11, 0.09), D87 (0.09, 0.06), D132 (0.18, 0.22), D135 (0.17, 0.19), D130 (0.16, 0.09), D68 (0.13, 0.15), D77 (0.18, 0.13), D121 (0.07, 0.17), D29 (0.19, 0.11), D63 (0.20, 0.04), D147 (0.25, 0.16), D14 (0.11, 0.13). The parameters of D1824 were not determined because of its straight

appearance. The closed squares represent the helical parameters of various types of flagellar filaments estimated from simulation data (11).

Reference

Shibata S, Alam M, and Aizawa S. 2005. Flagellar filaments of the deep-sea bacteria *Idiomarina loihiensis* belong to a family different from those of *Salmonella typhimurium*. *J. Mol. Biol.* **352**:510-516.