The assembly order of flagellar rod subunits in *Bacillus subtilis*

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Supplemental Material

Table S1. Plasmids

Plasmid	Genotype	
pAH25	*amyE*::spec erm amp	**(1)**
pAMB1	*amyE*::$P_{fla/che}$ cat amp	
pAMB3	*amyE*::$P_{fla/che}$-*flgC* cat amp	
pAMB4	*amyE*::$P_{fla/che}$-*fliE* cat amp	
pDG364	*amyE*::cat amp	**(2)**
pEV1	*amyE*::$P_{fla/che}$-*flgB* spec amp	
pEV2	*SUMO-flgB* amp	
pEV3	*SUMO-flgC* amp	
pEV4	*SUMO-flhO* amp	
pEV5	*SUMO-flhP* amp	
pKRH12	*SUMO-fliE* amp	
pTB146	*6His-SUMO* amp	**(3)**

Tabls S2. Primers

Primer	Sequence
3041	AGGAGGGATCCCGTTTACAAGATCAATTCAATGACG
3042	CTGCTGAATTCAGCTCTTGTCGTATCCATATTTGCT
3150	AGGAGGCTCTTCAGGTAGCTTATTTTCTGGAACGATAC
3151	CTCCTCTCGAGTCACCTGCTTAGCTCTTGTC
3152	AGGAGGCTCTTCAGGTACAGCTTTTCATAGCTTAAATGTTTC
3153	CTCCTCTCGAGCTCATTTAACGAACTAATAGAG
3154	AGGAGGCTCTTCAGGTTTAAAAGGATTATATACAGCAACA
3155	CTCCTCTCGAGGAATTGCTGTCTGACAAGCTC
3156	AGGAGGTCGACTCAGGTCAATGCTAACGGC
3157	CTCCTGGATCCCTGCTCGAATATGTGCTTGAA
4198	AGGAGGCTCTTCCGGTATGAATGTGATTAATGCAATTTCT
4199	AGGAGCTCGAGCCCCGGTCATTCCACTCTT
4773	CCTCCTGGGATCCGGCTATTGTTTACTTGATTTGTTGC
4775	CCTCCTGGATCCCCACTCTTTCTCTAAATCGTCAGAC
4797	AGGAGGCAATTGAGGTACTTATATCAAGGTACTAA
4798	CCTCCTGGATCCTAGGCTAGCATCGAATTCTCACCCTCAATATCCTTGTCGAG
4799	AGGAGGGAATTCGAACGGTAAATTTAATTCGCTGAA
4800	AGGAGGGAATTCGGGAATGCTGATGAAGGCGTTAG

Supplemental references

1. 1. **Guérout-Fleury AM, Frandsen N, Stragier P.** 1996. Plasmids for ectopic integration in *Bacillus subtilis*. Gene. **180**:57-61.

2. **Antoniewski C, Savelli B, Stragier P.** 1990. The *spoIIJ* gene, which regulates early developmental steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. J. Bacteriol. **172**:86-93.

3. **Bendezu FO, Hale CA, Bernhardt TG, de Boer PA.** 2009. RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*. EMBO J. **28**:193–204.

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Sen_FlgB   1 MLDRLDAALRFQQEALNLRAQRQEILAANIANADTPGYQARDIDFASELKKVMVRGREETGGVALTLTSSHHIPA-QAVS----SPAVDLLYRVP
Bbu_FlgB   1 -MNDFERSVDFSHRYLDVLSLRQSVISDNIANVDTPNFKRSKISFESELEKAFLNEDKND--LNLIKSSDKHLSGFKNLKYSDVKPH--RVLDHF
Bsu_FlgB   1 -MSLFSGTIQNLENALSRADIKQKVITNNIANIDTPNYKAKKVSFQNLLDQES-----SR--LEAIKTD------YRHVDFSDTDSNYSIVASGD

Sen_FlgB  91 DQPSLDGNTVDMDRERTQFADNSLKYQMGLTVLGSQLKGMMNVLQGGN
Bbu_FlgB  91 STMNNNGNNVDIDSEVKALVQNQMMYHLMTNVQAHYFKSINIVLK---
Bsu_FlgB  82 TSYQQNGNNVDVDKEMTELAQNQINYQALVERMNGKFNSLKTVLTGGK


Sen_FlgC   1 MALLNIFDIAGSALAAQSKRLNVAASNLANADSVTG----PDGQPYRAKQVVFQV--------------DAAPGQATGGVKVASVIESQAPEKLV
Bbu_FlgC   1 MGLFSSINVASTGLTAQRLRIDVISNNIANVSTSRT----PDGGPYRRQRIIFAPRVNNPYWKGPF-IPDYLDNGIGQGVRVASIEKDKSPLKLK
Bsu_FlgC   1 MTAFHSLNVSASALTAQRVRMDVVSSNLANMDTTRAKQVNGEWVPYRRKMVSLQSKGES--FSSILNSQMSGSGNAGNGVKVSKITEDDSDFNLV

Sen_FlgC  78 YEPGNPLA----DANGYVKMPNVDVVGEMVNTMSASRSYQANIEVLNTVKSMMLKTLTLGQ-
Bbu_FlgC  91 YDPAHPDSISSGDKKGYVELPNVNLVEEMVDMISASRAYEANSTVINSSKSMFRSALAILQG
Bsu_FlgC  94 YDPTDPDA----NAEGYVQKPNVDPLKEMVDLVSSTRSYEANVTAMNATKGMLMKALEIGK-


Sen_FlgF   1 MDHAIYTAMGAASQTLNQQAVTASNLANASTPGFRAQLNALRAVPVDGLSLA---------------TRTLVTASTPG-------ADMTPGQLDYT
Bbu_FlhO   1 MVRGIYTAASGMMAERRKLDTVSNNLANIDLIGYKKDLSIQKAFPEMLIRRLNDDGLYKFPKGHLETAPVVGKIGTGVEENEIYTVBEQGPLKTT
Bsu_FlhO   1 MLKGLYTATSAMIAQQRRTEMLSNNTANANTSGYKADQGSMRAFPEMLLSRIESKSPA----GT--SRTEIGSVNTGVYMQELKPLFTQGSLKST

Sen_FlgF  75 SRPLDVALQQD--------------GWLVVQAADGAEGYTRNGNIQVGPTGQLTI-QGHPVIG-EGGPITVPEGSEITIAADGTISALNP--GDPP
Bbu_FlhO  96 GNPLDLALTDQ--------------GFFVIQTSDG-ERYTRNGSFTIGKEGILVTKSGFPVLG-EKGYIYLKK-NNFKITPQGQVFHNSNFESDPK
Bsu_FlhO  90 DQPTDIALIENQVPMSAETNEKAALFYPVQTADG-IRYSKSSTFSLNENNQLTI-NGRPILSTDRQPITVDN-ENFTVSENGTVTTNGRTA----

Sen_FlgF 153 NTV----------APVGRLK--------LVKAEGNEVQRSDDGLFRLTAEAQAERGAVLAADPSIRIMSGVLEGSNVKPVEAMTDMIANARRFEM
Bbu_FlhO 175 RLVSEYENSWENYELLDTIRIVNFENPRFLKKQGNSLWIDTKTSGK---------AQEIDISLRPKIETETLEASNVNAVKEMVLMIEINRAYEA
Bsu_FlhO 178 ---------------GQIDVRMAEDVRNLKRDGNDLYSTADGNDLPSA----AGNNQVAYSL----KQGVSELSNVDVTSAYTEMTEAYRSFEA

Sen_FlgF 230 QMKVITSVDENEGRANQLLSMS
Bbu_FlhO 261 NQKTIQTEDSLLGKLINEIGKY
Bsu_FlhO 249 NQKVIQAYDKSMDKAANEIGKI


Sen_FlgG   1 MISSLWIAKTGLDAQQTNMDVIANNLANVSTNGFKRQRAVFEDLLYQTIRQPGAQSSEQTTLPSGLQIGTGVRPVATERLHSQGNLSQTNNSKDV
Bbu_FlgG   1 MMRALWTAASGMTAQQYNVDTIANNLSNVNTTGFKKIRAEFEDLIYQTHNRAGTPATENTLRPLPNGQVGHGFTKIAATQRIFEQGKMQSTNLLTDV
Bsu_FlhP   1 MLKGLYTATSAMIAQQRRTEMLSNNTANANTSGYKADQGSMRAFPEMLLSRIESKSPAGTSRTEIGSVNTGVYMQELKPLFTQGSLKSTDQPTDI

Sen_FlgG  96 AIKG-------------QGFFQVMLPDGTSAYTRDGSFQVDQNGQLVTAGGFQVQPAITIPANAL--SITIGRDGVVSVTQQGQAAPVQVGQLNL
Bbu_FlgG  96 AIEG-------------DGFYKILLPDGTYAYTRDGSFKIDSNRELVTSQGYKVLPNILFPEEYIQNSITISEEGIVSVKIDTSNEEPIELGQIEI
Bsu_FlhP  96 ALIENQVPMSAETNEKAALFYPVQTADG-IRYSKSSTFSLNENNQLTINGR-PILSTDRQPITVDNENFTVSENGTVTTNG------RTAGQIDV

Sen_FlgG 176 TTFMNDTGLESIGENLYIETQSSGAPNESTPGLNGAGLLYQGYVETSNVNVAEELVNMIQVQRAYEINSKAVSTTDQMLQKLTQL----
Bbu_FlgG 178 SRFINPAGLSAIGSNLFKETAGSGQEIAGIPGSEGMGRLRQGILEMSNVSIAEEMVTMIVAQRAYEINSKAIQTSDNMLGIANNLKRQ-
Bsu_FlhP 183 RMAEDVRNLKRDGNDLYSTADGNDLPSAAG-NNQVAYSLKQGVSELSNVDVTSAYTEMTEAYRSFEANQKVIQAYDKSMDKAANEIGKI


Sen_FlgE   1 --MSFSQAVSGLNAAATNLDVIGNNIANSATYGFKSGTASFADMFAGS----------------KVGLGVKVAGITQDFTDGTTTNTGRGLDVA
Bbu_FlgE   1 MMRSLYSGVSGLQNHQTRMDVVGNNIANVNTIGFKKGRVNFQDMISQSISCASRPTDARGGTNPKQVGLGMNVASIDTIHTQAGAFQSTQKASDLG
Bsu_FlgE   1 MLRSLYSGISGMKNFQTKLDVIGNNIANVNTVGFKKSRVTFKDMVSQTIACGSAAGATIGGTNSKQIGLGSSSGTIDTIHSTSATQSTGRTLDLA

Sen_FlgE  77 ISQNGFFRLVDSNGSVFYSRNGQFKLDENRNLVNM-QGMQLTGYPATGTPPT--IQQGANPAPITIPNTL-MAAKSTTTASMQINLNSTDPVPSK
Bbu_FlgE  96 VSGNGFFILKEG-KNLFYTRAGAFDVDSDRHLVNPANGMRIQCWMARDLEGEKVINTASDIEDLIIPIGDKEGAKSTKNVTFACNLDKRLPLIQE
Bsu_FlgE  96 IDGDGYFRIDTG-DGTAYTRAGNFYLDNTGTLVTGD------CYHVLNMNCGTI----------KIPT------------------------

Sen_FlgE 168 T--PFSVSDADSYNKKGTVTVYDSQGNAHDMNVYFVKT--KDNEWAVYTHDSSDPAATAPTTASTTLKFNENGILES------------------
Bbu_FlgE 190 GASPADIARG---TWVVNKSLYDSFGNVSVLELRVVKDLNTPNLWNATVLINGE------QNSNFTLGFDNEGALASLNGQPGQKGDILQIPITF
Bsu_FlgE 147 -----------------------------------------------------------DAQTFSICGSDGK--------------------

Sen_FlgE 241 ------------GGTVNITTGTINGATAATFSLSFLNSMQQNTGANNIVATNQNGYKPGDLVSYQINNDCTVVGNYSNEQEQVLGQIVLANFANN
Bbu_FlgE 276 NVLGANVGEVGEQQTVNLKLGTVG---------SYTDSITQFADSSSTKAIIQDGYGMGYMENYEIDQNGVIVGIYSNGIRRDLGKIALASFMNP
Bsu_FlgE 159 ---------------------------------------------------------VSIVDAEGKTQDGGQIGIVTFANS

Sen_FlgE 324 EGLASQGDNVWAATQASGVA-LLGTAGSGNFGKLTNGALEASNVDLSKELVNMIVAQRNYQSNAQTIKTQDQILNTLVNLR-
Bbu_FlgE 362 GGLAKSGDTNFVETSNSGQVR-ICETGLAGLGDIRSGVLEMANVDLAEQFTDMIVTQRGFQANAKTITTSDQLLQELVRLKN
Bsu_FlgE 183 DGLDKIGSNLYRESLNSGTASAANQPGDGGTGALKSGFLEMSNVDLTDEFTEMIVAQRGFQSNSKITTTSDEILQELVNLKR


Sen_FliE   1 MAAIQGTEGVIS-----QL------QATAMAARGQDTHSQSTVSFAGQLHAALDRISDRQAAARVQAEKFTLGEPGIALNDVMADMQKASVSMQM
Bbu_FliE   1 --MV-RTDAFFTKNNLVKKNPLHFDVNLFSSKSNAKDNDIKTFKDVLINSITDVNKSQLNVSKVTEQAILKPSSIDVHDVVIAMSKANMNLSI
Bsu_FliE   1 --MINAITPFSPQVQNTQNTQ------NATNQVNNSQKTDSSNQTSFSELLKNSISSLNESQVASDNMTNAL-AAGKDVNLDEVMIAAQKASISLTA

Sen_FliE  93 GIQVRNKLVAAYQEVMSMQV
Bbu_FliE  85 LKAVVERGVKAYQDIINIR-
Bsu_FliE  87 ATEFRNKAVEAYQEIMRMQM
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Figure S1: Genetic alignment of *B. subtilis* rod proteins. Sequences of *B. subtilis* FlgB, FlgC, FlhO, FlhP, FlgE, and FliE (Bsu) were aligned with homologous rod and hook proteins from *Salmonella enterica serovar Typhimurium* (Sen) and *Borrelia burgdorferi* (Bbu).
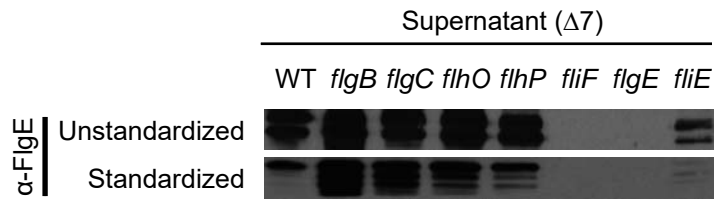
Figure S2: The *fliE* **mutant reduces flagellar type III secretion.** Western blot analyses of the hook protein FlgE in TCA precipitated supernatants. Precipitated pellets were resuspended to 100 OD units based on the OD_{600} of the culture at time of supernatant isolation. Samples were loaded with equal volumes (top panel) or standardized to a total protein concentration of 35 mg/ml and equally loaded (bottom panel). Samples were generated as described in Materials and Methods using the following strains: DK4537 (Δ7), DK4540 (Δ7 Δ*flgB*), DK4541 (Δ7 Δ*flgC*), DK4542 (Δ7 Δ*flhO*), DK4543 (Δ7 Δ*flhP*), DK4544 (Δ7 Δ*flgE*), DK4538 (Δ7 ΔfliF), and DK4539 (Δ7 ΔfliE).