Large ring polymers align FtsZ polymers for normal septum formation

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Content:

Table S1: *B. subtilis* strains used in this study.

Name	Relevant genotype	Reference
168	*trpC2*	Laboratory stock
CRK6000	*purA16 metB5 hisA3 guaB*	(Moriya et al., 1990)
MD133	*trpC2 ftsA*::*cat aprE*::P_{spac}-*ftsA kan*	(Ishikawa et al., 2006)
4181	*trpC2 amyE*::P_{xyl}-*sepF-gfp spc*	(Hamoen et al., 2006)
YK84	CRK6000 *sepF*::*cat*	This work
YK93	CRK6000 *amyE*::P_{xyl}-*sepF cat*	This work
YK107	CRK6000 *ftsA*::*erm amyE*::P_{xyl}-*sepF cat*	This work
YK146	CRK6000 *amyE*::P_{xyl}-*sepFA98V cat*	This work
YK150	CRK6000 *amyE*::P_{xyl}-*sepFF124S cat*	This work
YK179	CRK6000 *ftsA*::*erm amyE*::P_{xyl}-*sepFA98V cat*	This work
YK183	CRK6000 *ftsA*::*erm amyE*::P_{xyl}-*sepFF124S cat*	This work
YK204	CRK6000 *sepF*::*spc*	(Ishikawa et al., 2006)
YK206	CRK6000 *ftsA*::*erm*	(Ishikawa et al., 2006)
YK208	CRK6000 *aprE*::P_{ftsAZ}-*gfp-ftsZ spc*	(Ishikawa et al., 2006)
YK355	CRK6000 *sepF*::*cat amyE*::P_{xyl}-*sepFG135N-gfp spc*	This work
YK1040	*trpC2 ftsA*::*erm*	(Ishikawa et al., 2006)
YK1042	*trpC2 sepF*::*spc*	(Ishikawa et al., 2006)
YK1236	*trpC2 ftsA*::*cat aprE*::P_{spac}-*ftsA kan* Δ*sepF*::pMutin4	This work
YK1237	*trpC2 ftsA*::*cat aprE*::P_{spac}-*ftsA kan sepF*::pMutin4	This work
YK1238	*trpC2 ftsA*::*cat aprE*::P_{spac}-*ftsA kan sepFG135N*::pMutin4	This work

Hamoen, L.W., Meile, J.C., de Jong, W., Noirot, P. and Errington, J. (2006) SepF, a novel FtsZ-interacting protein required for a late step in cell division. *Mol Microbiol*, 59, 989-999.

Ishikawa, S., Kawai, Y., Hiramatsu, K., Kuwano, M. and Ogasawara, N. (2006) A new FtsZ-interacting protein, YlmF, complements the activity of FtsA during progression of cell division in *Bacillus subtilis*. *Mol Microbiol*, 60, 1364-1380.

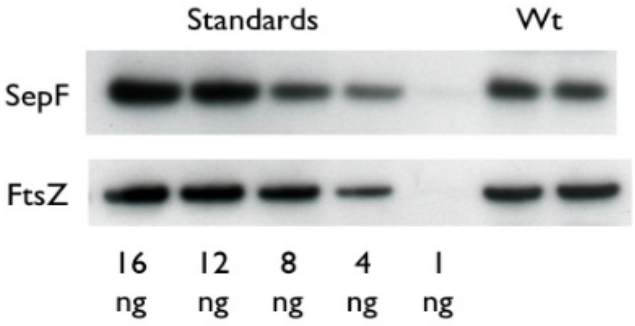
Moriya, S., Kato, K., Yoshikawa, H. and Ogasawara, N. (1990) Isolation of a *dnaA* mutant of *Bacillus subtilis* defective in initiation of replication: amount of DnaA protein determines cells' initiation potential. *EMBO J*, 9, 2905-2910.

Table S2: Primers used in this study. Forward and reverse primers are indicated by 'F' and 'R', respectively.

Name	Information	Sequence
Eg15	A98V F	CAGCATGACCAGGTGAAGCGGATTGTTG
Eg16	A98V R	CAACAATCCGCTTCACCTGGTCATGCTG
Eg23	F124S F	CGGCTCAGATATTTCCCTCTGCACGCCTGAC
Eg34	F124S R	GTCAGGCGTGCAGAGGGAAATATCTGAGCCG
Eg94	RF pMal-SepF F	AACAACCTCGGGATCGAGGGAAGGATGAAAG ATAAACTGAAAAACTTTTTC
Eg95	RF pMal-SepF R	CAGTGCCAAGCTTGCCTGTCATTACCACCTCT GATGTTCGTCTTCAGAT
sepF134F	Δ134 F	GTAGATGTATCATAAACAATTTCTGAG
sepF134R	Δ134 R	CTCAGAAATTGTTTATGATACATCTAC
sepFG135N-F	G135N F	CGTAGATGTATCTAACACAATTTCTGAG
sepFG135N-R	G135N R	CTCAGAAATTGTGTTAGATACATCTACG
LH224	pMutin-sepF F	AAAGAATTCTAAGAGACAGAGCGGGAATCTC
LH225	pMutin-sepF R	AAAGGATCCTTACCACCTCTGATGTTCGT

Fig. S1. (A) A Western blot of SepF and FtsZ, with titrations of purified SepF or FtsZ standards, and total protein from wild type cells (Wt). (B) Estimated cellular concentrations of SepF and FtsZ from different experiments (see Material and Methods for details). Cultures were grown at 30 °C or 37 °C. For calculations see Experimental Procedures.

A



B

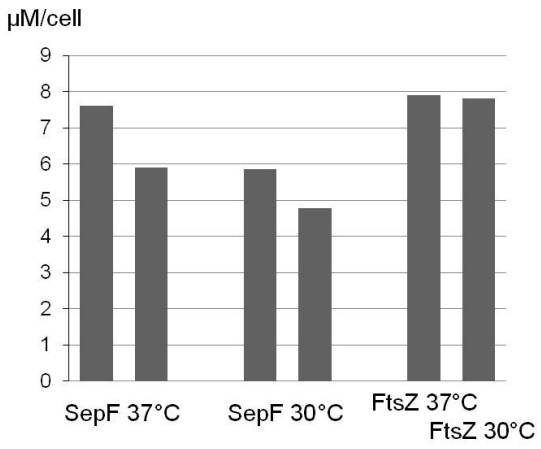


Fig. S2. SDS-PAA gel of one of the FtsZ pelleting assays. Samples were prepared in pH 6.5 polymerization buffer (50 mM MES pH 6.5, 50 mM KCl, 10 mM $MgCl_2$), or in pH 7.4 buffer (50 mM Tris-HCl pH 7.4, 300 mM KCl, 10 mM $MgCl_2$), using 10 µM FtsZ, 6 µM SepF and 1 mM GTP. FtsZ was incubated in the absence (-) or presence (+) of GTP and SepF (SepF). After incubation the samples were centrifuged and fractions of the supernatants (sup) and pellets (pel) were loaded onto a SDS-PAA gel. (B) SDS-PAA gel of pellet (pel) and supernatant (sup) fractions of 6 µM SepF in polymerization buffer pH 6.5 or pH 7.4 (without GTP).

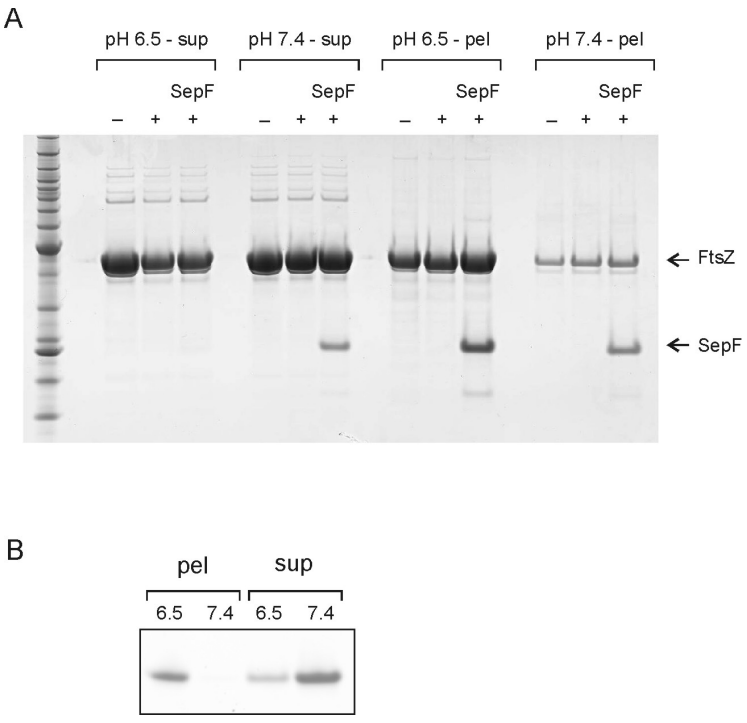


Fig. S3. Light scattering analyses of FtsZ polymerization under different buffer conditions, (A) 50 mM MES pH 6.5, 50 mM KCl, 10 mM $MgCl_2$, (B) 50 mM MES pH 6.5, 300 mM KCl, 10 mM $MgCl_2$, (C) 50 mM Tris pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, and (D) 50 mM Tris pH 7.4, 300 mM KCl, 10 mM $MgCl_2$. All reactions contained 10 μM FtsZ, and were initiated by the addition of 2 mM GTP after 1 minute equilibration. A 350 nm wavelength and 2.5 nm slit-width was used for excitation and emission.

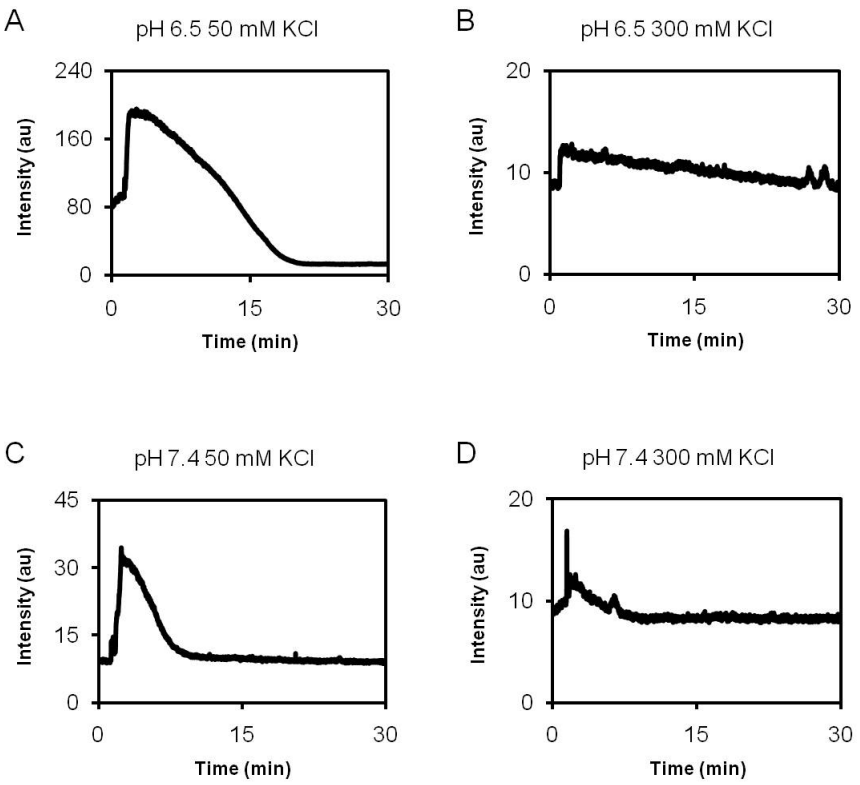


Fig. S4. Growth of FtsZ-SepF tubules. Compilation of different images taken over a 20 min time period. After 1 min no FtsZ-SepF tubules are observed, but SepF rings tend to cluster (central dark region). After 5 min, FtsZ-SepF tubules can be observed, which often seem to emerge from a cluster of SepF rings. After 20 min very long tubules (up to 5 μm) were observed. Scale bar for the 1, 5 and 10 min images is 100 nm, and for the 20 min images is 500 nm.

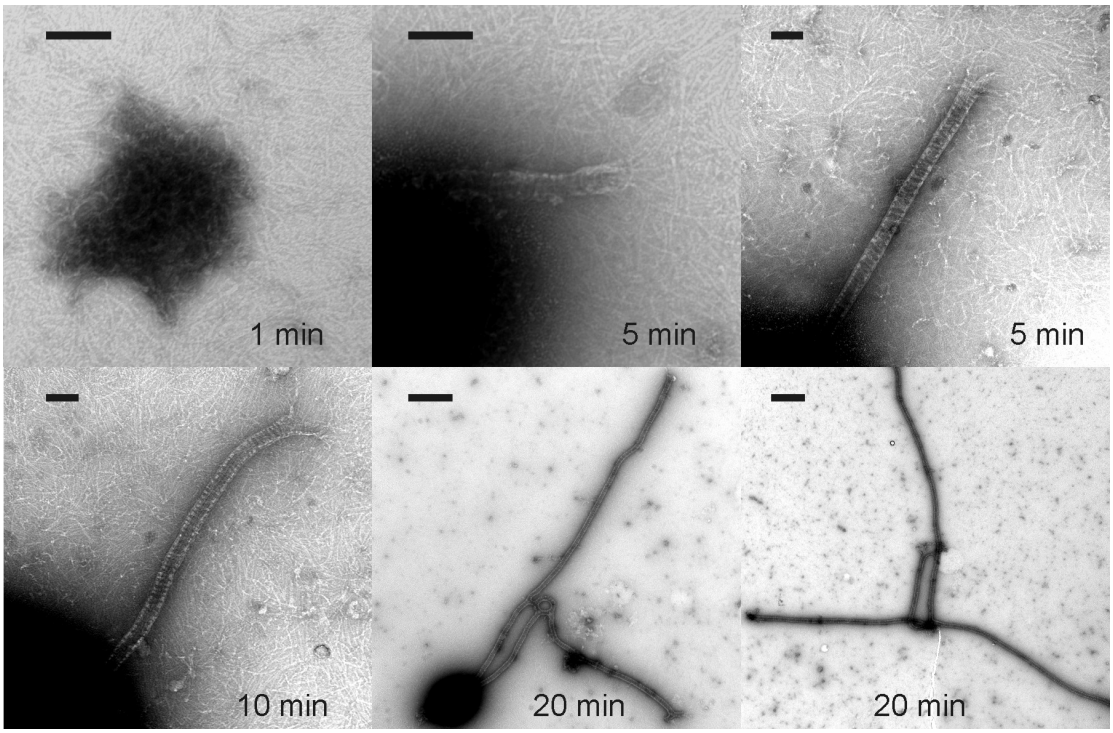


Fig. S5. EM images of negatively stained SepF mutants A98V (left) and F124S (right) mixed with FtsZ. Standard FtsZ polymerization conditions were used (50 mM Tris-HCl pH 7.4, 300 mM KCl, 10 mM $MgCl_2$, 1 mM GTP), and concentrations of 10 µM, and 6 µM for FtsZ and SepF, respectively (scale bar: 100 nm). Mutant A98V makes almost no rings (see main text), and no rings could be discerned in the EM images. Mutant F124S makes normal rings, and some are indicated by a white arrow.

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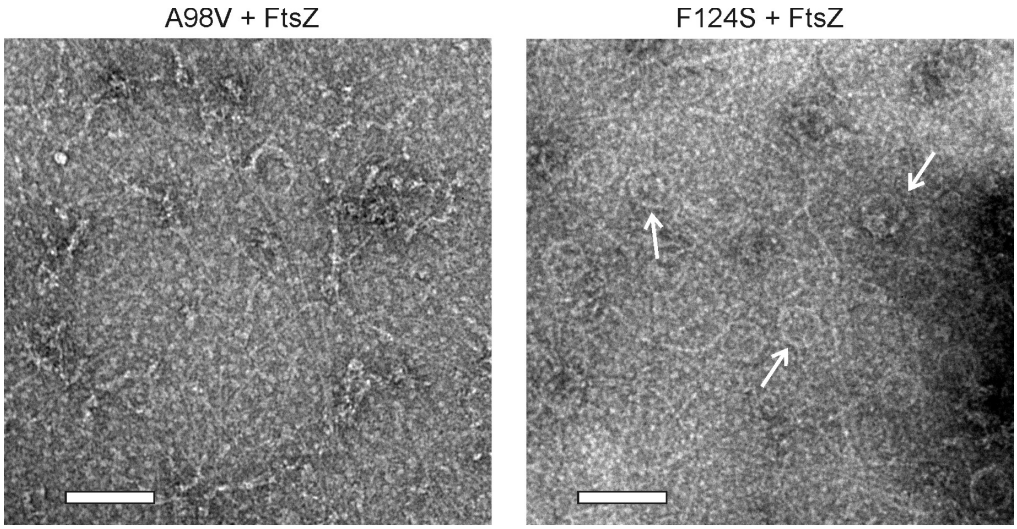

Fig. S6. Transmission electron micrographs of division septa in *B. subtilis* wild type (wt), Δ*ftsA*, and Δ*sepF* cells. For each strain two examples of negatively stained thin sections are shown. Scale bar represents 200 nm.