



LUND UNIVERSITY

Influence of core divisome proteins on cell division in *Streptomyces venezuelae* ATCC 10712

Cantlay, Stuart; Sen, Beer Chakra; Flårdh, Klas; McCormick, Joseph R.

Published in:
Microbiology (Reading, England)

DOI:
[10.1099/mic.0.001015](https://doi.org/10.1099/mic.0.001015)

2021

Document Version:
Peer reviewed version (aka post-print)

[Link to publication](#)

Citation for published version (APA):
Cantlay, S., Sen, B. C., Flårdh, K., & McCormick, J. R. (2021). Influence of core divisome proteins on cell division in *Streptomyces venezuelae* ATCC 10712. *Microbiology (Reading, England)*, 167(2).
<https://doi.org/10.1099/mic.0.001015>

Total number of authors:
4

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

**Influence of core divisome proteins on cell division in
Streptomyces venezuelae ATCC 10712**

Stuart Cantlay^{1,#}, Beer Chakra Sen², Klas Flärdh² and Joseph R. McCormick^{1,*}

¹ *Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA*

² *Department of Biology, Lund University, 223 62 Lund, Sweden*

[#]*Current address: Department of Biological Sciences, West Liberty University, West Liberty, WV 26074, USA*

**Corresponding author*

Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA

Tel: (+1) 412 396 4775

Email: mccormick@duq.edu

Running Title: Divisome genes in *S. venezuelae*

Keywords: divisome, septation, sporulation, spore, morphological development

24 **ABSTRACT**

25 The sporulating, filamentous, soil bacterium *S. venezuelae* ATCC 10712 differentiates under
26 submerged and surface growth conditions. In order to lay a solid foundation for the study of
27 development-associated division for this organism, a congeneric set of mutants was isolated,
28 individually deleted for a gene encoding either a cytoplasmic (i.e., *ftsZ*) or core inner membrane
29 (i.e., *divIC*, *ftsL*, *ftsI*, *ftsQ*, *ftsW*) component of the divisome. While *ftsZ* mutants are completely
30 blocked for division, single mutants in the other core divisome genes resulted in partial, yet similar,
31 blocks in sporulation septum formation. Double and triple mutants for core divisome membrane
32 components displayed phenotypes that were similar to the single mutants, demonstrating that the
33 phenotypes were not synergistic. Division in this organism is still partially functional without
34 multiple core divisome proteins, suggesting that perhaps other unknown lineage-specific proteins
35 perform redundant functions. In addition, by isolating an *ftsZ2p* mutant with an altered -10 region,
36 the conserved developmentally controlled promoter was also shown to be required for sporulation-
37 associated division. Finally, microscopic observation of FtsZ-YFP dynamics in the different
38 mutant backgrounds led to the conclusions that the initial assembly of regular Z rings does not *per*
39 *se* require the tested divisome membrane proteins, but that stability of Z rings is dependent on the
40 divisome membrane components tested. The observation is consistent with the interpretation that
41 Z ring instability likely results from and further contributes to the observed defects in sporulation
42 septation in mutants lacking core divisome proteins.

43 INTRODUCTION

44 Since the 1960s, *Streptomyces coelicolor* A3(2) has been used as the model system to study the
45 filamentous sporulating soil bacteria in the genus *Streptomyces* [1]. The streptomycetes are true
46 mycelial organisms of ecological importance, and are responsible for the synthesis of many
47 important biologically active compounds, including a wide range of antibiotics [2]. To facilitate
48 dispersal in the environment, and to enable long-term survival, streptomycetes produce semi-
49 dormant spores. Spores are formed as part of a complex developmental life cycle, in which the
50 streptomycete initially grows vegetatively as branching hyphae while nutrients are available,
51 forming a vegetative mycelium, also referred to as substrate mycelium. Eventually, specialized
52 spore-forming aerial hyphae emerge on the colony surface. The apical parts of such aerial hyphae
53 are partitioned into chains of prespores via synchronous formation of several tens of cell division
54 septa in each hypha, and thereafter prespores mature to form pigmented and thick-walled spores
55 with condensed nucleoids and a hydrophobic outer surface layer, before being released into the
56 environment (reviewed in e.g. [3-5]). When conditions are favorable, spores germinate and grow
57 to form a new mycelium. The formation of aerial mycelium and spores is governed by a regulatory
58 cascade of transcription factors that has been studied mainly in *S. coelicolor*, but more recently
59 much progress has been made in mapping the developmental regulatory networks in the new model
60 organism *Streptomyces venezuelae* [3-5].

61 Cell division and its regulation are of central importance in the *Streptomyces* life cycle, which
62 involves two distinct types of division. Both types are formed by a typical bacterial cell division
63 machinery, organized by the tubulin homolog FtsZ [6, 7]. In vegetatively growing hyphae, cell
64 division is infrequent and gives rise to widely-spaced hyphal cross-walls. Intriguingly, vegetative
65 hyphae can grow even in the absence of such cross-walls and cell division is not essential for
66 proliferation of these organisms; the key cell division gene *ftsZ* is dispensable for growth and
67 viability in *S. coelicolor* and *S. venezuelae* [8, 9]. The *ftsZ*-null mutants do not make cross-walls
68 at all, but can still grow as branching hyphae. On the other hand, cell division is absolutely essential
69 for spore formation. The septa that divide aerial hyphae into prespore compartments are formed
70 by the same *ftsZ*-dependent core cell division machinery as the vegetative cross-walls. However,
71 sporulation septa differ from hyphal cross-walls. Structurally, sporulation septa are thicker,

72 eventually leading to full constriction and separation of daughter cells, and sporulation septation
73 is subject to spatial and temporal regulation [10].

74 Cell division genes are developmentally regulated and directly controlled by several of the key
75 transcriptional regulators that govern morphological differentiation and sporulation. For example,
76 *ftsZ* has a developmentally-regulated promoter, *ftsZ2p*, that is critical for spore formation [11].
77 This promoter is repressed by the master regulator BldD in complex with cyclic di-GMP;
78 c-di-GMP-bound BldD negatively controls many important genes related to aerial mycelium
79 formation and sporulation [12, 13]. In *S. venezuelae*, both the *ftsZ2p* promoter and promoters for
80 cell division genes *ftsW*, *ftsK*, and *sepF2* are activated by the proteins WhiA and WhiB, which
81 presumably act as a complex that controls a large regulon of genes involved in spore formation
82 [14, 15]. In addition, *ssgB* and, indirectly, *ssgA* are controlled by the developmental regulator
83 BldM [16]. SsgA and SsgB belong to an actinobacteria-specific protein family and affect selection
84 of septation sites in *S. coelicolor* [17]. Finally, two dynamin-like proteins are involved in septation
85 specifically in sporulating aerial hyphae, and developmental upregulation of the corresponding
86 genes, *dynA* and *dynB*, depends on the sporulation-specific GntR-family regulator WhiH in *S.*
87 *venezuelae* [18].

88 The bacterial cell division process is primarily controlled at the level of assembly of FtsZ into
89 cytokinetic polymers and their formation of a ring-shaped pattern, the Z ring, which serves to
90 recruit and organize most other proteins involved in cell constriction and septum formation [19-
91 21]. Interestingly, streptomycetes, as well as Actinobacteria in general, lack obvious homologs of
92 most of the proteins that are known to regulate Z-ring formation, including MinC, MinD, SulA,
93 Noc, or to stabilize FtsZ polymers or tether them to the cytoplasmic membrane, like FtsA and
94 ZipA [6, 7]. The exception is SepF, which links FtsZ to membranes and facilitates Z-ring formation
95 in Gram-positive bacteria like *Bacillus subtilis* [22], and which is present as three homologues in
96 *S. venezuelae* [18]. The regulation of Z-ring assembly in *Streptomyces* remains poorly understood.
97 During sporulation, SsgA and SsgB proteins have been reported to mark the sites of Z-ring
98 formation in *S. coelicolor* [17], and DynA and DynB are recruited to septation sites and help
99 stabilize Z rings in *S. venezuelae* [18]. DynA and DynB interact with each other, and DynB also
100 interacts with both SsgB and one of the three SepF proteins in *S. venezuelae* [18]. Analyses with
101 two-hybrid systems have further suggested that the three SepF proteins interact with each other,

102 SepF interacts with FtsZ, and SepF2 interacts with both DynB and SsgB [18], indicating a
103 sophisticated protein interaction network affecting Z-ring assembly. Additional proteins have been
104 suggested to affect this critical step in cell division, including SepG and CrgA, but details still
105 remain unclear [23, 24].

106 Once formed, the Z ring recruits further division proteins that collectively are referred to as the
107 divisome. Present in streptomycetes are orthologues of conserved divisome proteins FtsQ, FtsL,
108 and DivIC(FtsB), which are known to form a complex with a structural and/or regulatory role in
109 *Escherichia coli* and *B. subtilis* [19, 25, 26], and FtsW and FtsI, which encode a peptidoglycan
110 transglycosylase of the shape, elongation, division, and sporulation (SEDS) family, and a cognate
111 penicillin-binding protein with transpeptidase activity, respectively [27]. In addition, the DNA-
112 translocase FtsK and ABC-transporter proteins FtsEX are encoded by streptomycete genomes [7].
113 Genetic studies in *S. coelicolor* show that none of these proteins are essential for growth or
114 viability, which is consistent with the finding that cell division is dispensable in streptomycetes.
115 However, although mutants for *ftsQ*, *ftsL*, *divIC*, *ftsW*, and *ftsI* are largely defective in spore
116 formation, none of these genes are absolutely needed for cell division, with all mutants being able
117 to form hyphal cross-walls and some sporulation septa [28-31]. For the last four genes, the mutant
118 phenotypes were found to be conditional, leading to suppression of the septation defect on minimal
119 medium or low osmolarity medium. Mutants lacking *ftsK* or *ftsEX* show apparently regular
120 sporulation septation, but *ftsK* mutants have a defect in chromosome stability, presumably related
121 to the role of FtsK in clearing trapped chromosomes from the closing septa [7, 32-34].

122 The fact that *Streptomyces* cell division is non-essential, developmentally regulated, disconnected
123 from vegetative growth, and involves previously unknown mechanisms for control of septum
124 formation, make streptomycetes attractive model systems to study the division process and its
125 regulation [6, 7]. This distinction is further accentuated by the recent development of live cell
126 imaging systems in the new model organism *S. venezuelae* that allow time-lapse visualization of
127 the cell division in great detail through the entire life cycle [3, 35]. In order to further establish *S.*
128 *venezuelae* as a cell division model system, we report the isolation and characterization of null
129 mutants for key cell division genes *ftsZ*, *ftsQ*, *ftsL*, *divIC*, *ftsW*, and *ftsI* for this organism, and we
130 clarify the effect of late divisome components on assembly of Z rings and cell division in *S.*
131 *venezuelae*.

132 **METHODS**

133 **Bacterial strains and growth conditions**

134 The *S. venezuelae* strains used in this study were derived from *S. venezuelae* strain ATCC 10712,
135 acquired from Dr. Colin Stuttard (Dalhousie University, Halifax, Canada) (Table S1). *S.*
136 *venezuelae* strains were cultivated at 30°C on maltose yeast extract medium (MYM) agar plates
137 or in MYM liquid medium [36], as described by Bush *et al.* [14]. *S. venezuelae* transconjugants
138 were selected on either MYM or R2S agar after interspecies conjugation, as described previously
139 [37]. Culture conditions and antibiotics followed previously described procedures for
140 streptomycetes [38]. *E. coli* strain TG1 was used for cloning, construction, and propagation of
141 vectors [39]. *E. coli* strain BW25113/pIJ790 [40-43] was used to create cosmid derivatives
142 containing insertion-deletion mutations. *E. coli* strain ET12567/pUZ8002 was used for
143 mobilization of *oriT*-containing cosmids and plasmids into *S. venezuelae* [37, 38]. *E. coli* strain
144 BT340 was used to express yeast Flp recombinase in *E. coli* to excise antibiotic resistance markers
145 flanked by *FRT* sites [44]. Culture conditions, antibiotic concentrations and genetic manipulations
146 generally followed those previously described for *E. coli* [39].

147

148 **Plasmids and general DNA techniques**

149 Plasmids and cosmids used in this study are listed in Table S2. DNA restriction and modifying
150 enzymes were used according to the manufacturer's recommendation (New England BioLabs).
151 Phusion DNA polymerase (Thermo Fisher Scientific) was used according to the manufacturer's
152 instructions. *S. venezuelae* total DNA preparations were obtained using the Wizard genomic DNA
153 purification kit (Promega). λ RED-mediated recombineering, modified for *Streptomyces*, was used
154 in *E. coli* to replace *S. venezuelae* genes on cosmids with mutagenic linear DNA cassettes [43].
155 Apramycin-resistance gene cassette [*oriT acc(3)IV*] was amplified by PCR from plasmid pIJ773.
156 The oligonucleotide primers used in this study are listed in Table S3. When necessary, the *bla* gene
157 of the cosmid backbone was replaced by recombineering with a *bla* homology-flanked *oriT*
158 *acc(3)IV*-cassette from pIJ799. Plasmid pMS82 was used to create genetic complementation
159 plasmids for site-specific integration in the chromosome at the Φ BT1 attachment site [45]. DNA

160 sequences of unmarked in-frame deletions were verified using BigDye cycle sequencing analyzed
161 on an ABI 3130 Prism Genetic Analyzer (Applied Biosystems).

162

163 **Isolation of strains mutant for cell division genes**

164 Using PCR product-directed recombineering of cosmid inserts [43], insertion-deletion mutations
165 were created in which all or crucial portions of the coding regions of *S. venezuelae* division genes
166 were replaced with an apramycin-resistance cassette (*oriT acc(3)IV*) from pIJ773 (Table S2). Care
167 was taken when designing the 3' endpoints of in-frame deletions to minimize the potential polar
168 effects on expression of the downstream co-transcribed gene(s). Mutagenized cosmids were
169 confirmed by restriction enzyme digestion and PCR amplification with primers flanking the
170 introduced mutations. These mutagenized cosmids were introduced into *S. venezuelae* by
171 interspecies conjugation and marked null mutants, generated by double homologous
172 recombination events, were identified among primary transformants by their apramycin-resistant,
173 kanamycin-sensitive phenotypes. Genomic DNA from mutant candidates was analyzed by PCR
174 amplification using primers flanking the mutations.

175 Most mutations were designed to introduce unique *XbaI* and *SpeI* sites flanking the *oriT acc(3)IV*
176 cassette that was inserted into cosmids to generate the marked insertion-deletions. These restriction
177 sites facilitated the isolation of unmarked deletion mutant strains. The mutagenized cosmids were
178 digested with *XbaI* and *SpeI* and re-ligated, removing the *oriT acc(3)IV* cassette, leaving a 6 bp in-
179 frame scar with the sequence of ACTAGA. Alternatively, the antibiotic-resistance cassette was
180 removed by site-specific recombination resulting in an 81-bp *frt* scar for unmarked *ftsZ* and *ftsI*
181 mutations. Subsequently, a linear *oriT acc(3)IV* cassette was used to replace the *bla* gene on the
182 cosmid backbone allowing conjugation into *S. venezuelae* and selection of exconjugants by
183 apramycin resistance marker in the vector backbone. Primary exconjugants generated by a single
184 homologous recombination incident were screened by PCR for gene conversion events in which
185 resident wild type alleles were replaced with the introduced unmarked mutagenized ones.
186 Generally, 5-10% of exconjugants had undergone gene conversion events. Exconjugants that were
187 homozygous for the mutant allele were re-streaked without selection to allow loss of integrated
188 cosmids and progeny colonies were screened for apramycin sensitivity, indicating intramolecular
189 homologous recombination events and the loss of the cosmid. All of the mutants chosen for further

190 characterization were checked by PCR amplification from genomic DNA with primers flanking
191 the introduced mutation to confirm the presence of only the unmarked deletion allele. Double
192 mutants were constructed by introducing marked insertion-deletion mutations into unmarked
193 single mutants, as described above for isolating single mutants. A triple mutant for *ftsL*, *ftsQ* and
194 *divIC* was isolated in a similar fashion from an unmarked double mutant strain. A double mutant
195 strain for the adjacent *ftsL* and *ftsI* genes was obtained by combining recombineering primers used
196 for single mutation isolation (i.e., using the 5' *ftsL* primer and the 3' *ftsI* primer). The resulting
197 Δ *ftsIL::apra* mutation was introduced into the chromosome in the same way as for single mutant
198 isolation.

199 For generation of a non-sporulating strain by manipulating the developmentally controlled *ftsZ2p*,
200 the TAGTGT residues of the -10 motif on cosmid Sv-4-G01 were replaced with an *oriT acc(3)IV*
201 cassette flanked by introduced *SpeI* and *XbaI* sites. Restriction digestion of the mutagenized
202 cosmid with *SpeI* and *XbaI* and re-ligation left ACTAGA in place of the native -10 sequence.
203 Exconjugants were selected as described above for unmarked mutations and identified by a PCR
204 analysis using oligonucleotides specific for each of the two promoter sequences.

205

206 **Construction of genetic complementation plasmids**

207 For genetic complementation, a series of DNA fragment inserts were generated from cosmids by
208 restriction digestion or amplification by PCR (Fig. 1a and Fig. S8) and cloned into site-specific
209 integration vectors pMS82. The resulting plasmids were introduced into *S. venezuelae* mutant
210 strains by conjugation and integrated *in trans* into the chromosome at the Φ BT1 attachment site.

211

212 **Construction of strains expressing fluorescent FtsZ-YPet fusion proteins**

213 Plasmid pKF351, carrying and *ftsZ-ypet* fusion in a vector that integrates at the Φ C31 attachment
214 site [46], was introduced into relevant mutants by interspecies conjugation, as described above.

215

216 **Microscopy**

217 For phase-contrast microscopy, bacteria were grown as confluent patches on MYM agar. Cover
218 slips were touched to the surface of sporulated patches and material lifted was mounted on pads of
219 1% agarose in PBS. Samples were visualized using a Nikon Eclipse E400 with a Nikon 100x 1.25
220 NA oil immersion objective and a MicroPublisher 5.0 RTV high resolution CCD camera
221 (Qimaging).

222 For staining of cell wall and nucleoids, cultures were grown on MYM agar or in MYM liquid
223 medium, and samples were fixed in ice-cold methanol for 5 minutes, washed twice in PBS and
224 mounted in 100 $\mu\text{g ml}^{-1}$ propidium iodide (Molecular Probes) and 10 $\mu\text{g ml}^{-1}$ WGA-FITC
225 (Molecular Probes) in 50% glycerol. Fixed and stained samples were then spotted onto pads of 2%
226 agarose in PBS and sealed with petroleum jelly. Fluorescence imaging was done with a Leica SP2
227 TCS confocal microscope using a Leica 63x 1.4 NA glycerin immersion objective.

228 In order to visualize fluorescent FtsZ-YPet fusion protein, cells were grown in liquid MYM,
229 harvested and fixed with 2.28% formaldehyde and 0.018% glutaraldehyde, washed in PBS, and
230 mounted on 1% agarose in PBS. To follow FtsZ dynamics, microfluidics-based time-lapse
231 microscopy was performed using the CellASIC ONIX system and B04A-03 microfluidic plates
232 (Merck Millipore), as described previously [35, 47]. The live-cell time lapse experiments were
233 repeated twice for each strain. Imaging was performed on a Zeiss AxioObserver.Z1 microscope
234 with Zeiss Plan-Apochromat 100 \times /1.4 Oil Ph3 objective, ZEN software (Zeiss) and an ORCA
235 Flash 4.0 LT camera (Hamamatsu). Images and movies were processed using ImageJ/Fiji [48].

236 For Transmission Electron Microscopy (TEM), cells were grown as lawns on MYM agar and fixed
237 in 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) and incubated for 1 h in 2% osmium
238 tetroxide. Cells were dehydrated by successive transfer in 5 steps from 50% to 100% ethanol. Cells
239 were washed in propylene oxide and then in a 1:1 solution of propylene oxide and Spurr's before
240 being incubated in Spurr's resin overnight at 60°C. Thin sections were stained with 2% uranyl
241 acetate and 1% lead citrate. Samples were visualized using a JEOL JEM-1210 equipped with a
242 Hamamatsu Orca-HR CCD camera.

243 RESULTS AND DISCUSSION

244 Generation of *ftsZ*-null mutants

245 The earliest acting divisome protein FtsZ is required for cell division and viability in most bacteria.
246 Previously, it had been demonstrated that *ftsZ*-null strains could be isolated for *S. coelicolor* [8].
247 However, it was not clear whether this would be a general property of streptomycetes. Therefore,
248 the procedure for isolating a deletion-insertion mutation for *S. coelicolor ftsZ* was replicated in
249 *S. venezuelae* by replacing 844 bp, starting 16 bp upstream of the *ftsZ* start codon, with an
250 apramycin-resistance cassette (Fig. 1a). The mutants described in this paper were isolated in *S.*
251 *venezuelae* ATCC 10712 acquired from Dr. Colin Stuttard (Dalhousie University, Halifax,
252 Canada). In three independent experiments, we were able to isolate *S. venezuelae* null mutants for
253 *ftsZ*. However, in contrast to the majority of the other cell division mutants isolated in
254 *S. venezuelae* (described below), obtaining the *ftsZ*-null mutants was not as straightforward. The
255 initial *ftsZ*-null mutant colonies grew very slowly after selection on conjugation plates, therefore,
256 waiting up to 8 days before picking colonies was necessary to identify these mutants as “specks”
257 or “flecks” on the agar surface. These primary mutant colonies on conjugation plates did not
258 increase in size upon prolonged incubation, nor did they sector. Subsequently, mutants were re-
259 streaked several times on selective media. *ftsZ*-null mutants were unhealthy and grew slowly on
260 the nalidixic acid-containing medium used for counter-selection of the conjugation donor *E. coli*,
261 perhaps contributing to the difficulty when isolating them initially and, similarly, when introducing
262 an empty vector as control for genetic complementation studies (see below). Once nalidixic acid
263 counter-selection was no longer needed, the single colonies were uniform in appearance and the
264 phenotype was stable. Colonies of the purified *ftsZ*-null strains appeared on plates at similar
265 incubation times relative to the wild type, however, plating efficiency was much lower (Figs. S1
266 and S2); mature colonies were smaller and took much longer to develop an aerial mycelium, as
267 judged by the surface of the colonies becoming white. Isolated independent strains had similar
268 microscopic phenotypes and the insertion-deletion mutation of *ftsZ* was confirmed by PCR from
269 genomic DNA. One representative strain was picked for further analysis (DU500). As expected,
270 western blot analysis verified that FtsZ was not detected in a whole cell extract from DU500 (Fig.
271 S3). As anticipated, phase-contrast and TEM microscopic analyses of the *ftsZ*-null mutant grown
272 on agar showed that aerial hyphae did not differentiate to produce spores and the vegetative hyphae

273 were devoid of the normal cross-walls (Figs. 1b and c). The *ftsZ*-null mutant was also completely
274 blocked in both cell division and spore production when grown in liquid cultures and little cell
275 material accumulated for the mutant under these growth conditions (data not shown). To confirm
276 that the observed division phenotype was the result of the introduced mutation, genetic
277 complementation studies were carried out. A restriction fragment containing *ftsQ*, *ftsZ* and the
278 native *ftsZ* promoters in the intergenic region between the genes was integrated at the chromosomal
279 *attΦBT1* site (pJS8; Fig. 1a). The complementation vector rescued the division phenotype of the
280 *ftsZ*-null mutants, as judged by phase-contrast microscopy (Fig. 1b), as well as restored growth
281 and colony size on agar medium (Figs. S2 and S3). We conclude that the deletion-insertion
282 mutation is not polar on downstream gene expression, and FtsZ-dependent cell division is
283 dispensable for growth and viability of *S. venezuelae*. Nonetheless, an unmarked *ftsZ*-null strain
284 was also generated and it had an identical microscopic phenotype as the insertion-deletion mutant,
285 but was not used further in this study (DU665, data not shown). The fact that an unmarked null
286 mutant can be isolated by gene conversion (see Methods) argues that the *ftsZ* mutants are difficult
287 to distinguish from background on primary conjugation plates, not that they can only be isolated
288 by very strong selection for marker replacement by double homologous recombination.

289 Using the same procedure that is described above, we have also isolated an *ftsZ*-null mutant
290 (DU669) in the *S. venezuelae* strain NRRL B-65442 obtained from Dr. Mark Buttner (John Innes
291 Centre, Norwich, UK) [49]. The mode of growth of this *ftsZ*-null mutant DU699 (NRRL B-65442
292 background) in the absence of cell division and hyphal cross-walls has been described elsewhere
293 [9]. As observed for other cell division mutants (data not shown), the macroscopic and microscopic
294 phenotypes of *ftsZ*-null mutants in the two independent wild type backgrounds were essentially
295 indistinguishable and could be genetically complemented, revealing no overt differences at the
296 phenotypic level between the *S. venezuelae* parent strains obtained from different sources. All
297 experiments described in the rest of this paper were carried out in the ATCC 10712 strain
298 background. (Nonetheless, about a dozen core divisome mutant strains were also isolated in the
299 NRRL B-65442 background. Their strain designations and genotypes are listed in Table S1.)

300

301 **The developmentally regulated promoter of *ftsZ* is required for sporulation-associated cell**
302 **division in *S. venezuelae***

303 For *S. coelicolor*, three promoters for *ftsZ* have been mapped to the 288 bp intergenic region
304 between *ftsZ* and *ftsQ*, and one of them, *ftsZ2p*, is developmentally regulated [11]. It has been
305 shown that BldD, a transcriptional regulator that plays a key role in *Streptomyces* development,
306 binds to the developmentally-regulated *ftsZ2p* promoter and acts to repress expression of *ftsZ*
307 during vegetative growth [12]. In *S. venezuelae* it has been shown that expression of *ftsZ* is
308 dependent on WhiA and WhiB, which are transcriptional regulators required for the transition from
309 growth of aerial hyphae to sporulation [14, 15]. ChIP experiments indicated that a WhiA binding
310 target lies around 158 bp from the predicted start codon of *ftsZ* [14], and in this region is a sequence
311 that is identical to the -10 region TAGTGT of the *S. coelicolor* *ftsZ2p* [11] and *S. griseus* P_{spo} [50].
312 The intergenic region upstream of *ftsZ* is highly conserved between *S. coelicolor* and *S. venezuelae*
313 with sequence conservation at the three mapped promoter regions, including the *ftsZ2p* promoter
314 (Fig. S4a). To test whether the importance of this developmental promoter for sporulation was also
315 retained in *S. venezuelae*, a strain was generated that was mutant for this presumed *ftsZ2p* promoter
316 region. In the unmarked mutant, the TAGTGT residues at the -10 region of this presumed promoter
317 were changed to ACTAGA (Fig. 4b). The resulting strain (DU523) had reduced plating efficiency
318 compared to the wild type, but grew robustly and formed an abundant aerial mycelium (Fig. S1).
319 However, the *ftsZ2p* mutant was unable to efficiently convert the aerial hyphae to spore chains
320 during growth on solid medium, and mature spores were observed much less frequently compared
321 to the wild type. Instead, longer spore-like compartments of irregular length were produced from
322 aerial hyphae (Fig. 1b). Thus, this promoter mutant can form functional division septa that result
323 in complete division events with cell separation that lead to formation of spore-like aerial hyphal
324 fragments. However as shown by the absence of regularly septated spore chains, the mutant has a
325 greatly reduced frequency of cell division compared to wild type. The phenotype of the
326 *S. venezuelae* *ftsZ2p* mutant is consistent with a failure to up-regulate the expression of *ftsZ*, and
327 this up-regulation is required for developmentally-associated cell division, similarly to what has
328 been observed in *S. coelicolor* and *S. griseus* [11, 50].

329

330 **Null mutants for core divisome genes**

331 In order to clarify the roles of some of the conserved core divisome proteins for *S. venezuelae*,
332 unmarked in-frame null mutants for *ftsQ* (strain DU629), *divIC* (strain DU613), *ftsL* (strain

333 DU520), *ftsW* (strain DU521) and *ftsI* (strain DU679) were isolated (Fig. 1a). These genes are
334 broadly conserved among bacteria and their products are membrane proteins required for
335 coordinating the cytoplasmic Z ring with the peptidoglycan synthesis machinery [19, 25]. In other
336 bacteria, FtsQ, FtsL and DivIC form a subcomplex that is recruited to the divisome. A 1 MDa
337 complex containing those proteins, along with FtsZ, has been identified for *E. coli* [51]. Likewise,
338 FtsI and FtsW form a subcomplex involved in septal peptidoglycan synthesis as a transpeptidase
339 and transglycosylase, respectively [27]. While it has not been demonstrated directly for
340 *Streptomyces*, it is reasonable to expect that the protein subcomplexes are conserved.

341 Since most of the core divisome genes are part of the complex *dcw* gene cluster (Fig. 1a) and are
342 likely co-transcribed with other cell wall biosynthetic genes, unmarked in-frame deletions were
343 generated to avoid polar effects on downstream genes. The mutants were readily isolated and
344 showed consistent macroscopic and microscopic phenotypes that were strikingly similar to one
345 another on MYM agar, but were distinct from the *ftsZ*-null and *ftsZ2p* mutants. In contrast to the
346 *ftsZ*-null mutant, plating efficiency was unaffected in these mutants (Fig. S1), suggesting that
347 vegetative cross-wall formation was not severely impaired. Likewise, aerial mycelium
348 development was essentially unaffected, however, aerial hyphae were not efficiently converted
349 into chains of spores. By 48 hrs of incubation on solid medium, the majority of aerial mycelium
350 had been converted into spores for the wild type. For the *ftsQ*, *ftsL*, *divIC*, *ftsW* and *ftsI* null
351 mutants, a mixture of spores, hyphal fragments and frequent lysed compartments were observed
352 (Figs. 1b and S5). Aerial hyphae often contained frequent and regularly-spaced constrictions,
353 reminiscent of sporulation septa (Fig. 1b). Some separated spores were produced and fragments of
354 varying lengths were also observed for each of these mutants, showing that the products of these
355 genes are not absolutely required for cell division for *S. venezuelae* (Figs. 1b and S5). Each
356 mutation was genetically complemented using site-specific integration plasmids with inserts
357 shown in Figure 1a. The complemented strains sporulate similar to the wild type parent indicating
358 that the phenotypes were associated with the introduced null mutations (Figs. 1b and S1).
359 Combining *ftsQ*, *divIC* and *ftsL* mutations as either double mutants or as a triple mutant did not
360 have a synthetic effect on the observed phenotypes, as judged by phase-contrast microscopy (Fig.
361 2), suggesting that the loss of all the parts of the putative subcomplex formed by their gene products
362 is no more deleterious than the loss of any one component. This result is consistent with the
363 interpretation that missing any one component must inactivate the remainder of the tripartite

364 complex. In addition, a double mutant lacking both *ftsW* and *ftsI* was also constructed and the
365 phenotype was indistinguishable from the individual *ftsW* and *ftsI* mutants (Fig. 2), suggesting that
366 the loss of both parts of the putative subcomplex formed by their gene products is no more
367 deleterious than the loss of either one component. Finally, deleting both adjacent *ftsL* and *ftsI* genes
368 together resulted in a mutant with a similar phenotype to an *ftsI* single mutant (Fig. 2), indicating
369 that removal of parts of both putative divisome subcomplexes is no more deleterious than the loss
370 of one part. The similarity of the core mutant phenotypes and lack of synergism when combining
371 divisome mutations seems to support a model where there is no apparent hierarchy of assembly of
372 the core divisome components in *S. venezuelae*. Further experimentation will be needed to clarify
373 the situation and define each contribution.

374 Spores could be isolated from the aerial mycelium of surface-grown cultures despite the fact that
375 development-associated division was impaired for core divisome single mutants with a reduction
376 in the number of spores produced relative to the wild type. In order to quantify the severity of
377 reduction in sporulation-associated division in these core divisome mutants, measurements were
378 made of mature spores and hyphal fragments harvested in a typical fashion from agar plates after
379 4 days incubation. For the wild type, the material harvested consisted almost entirely of spores
380 with an average length of 1.00 (± 0.23) μm (Fig. S6). In contrast, the average lengths of the spores
381 and hyphal fragments for the *ftsQ*, *divIC* and *ftsL* mutants were similar at 2.13 (± 1.76) μm , 2.75
382 (± 2.67) μm and 2.49 (± 3.92) μm , respectively, suggesting that in aerial hyphae when development-
383 associated division resulting in cell separation occurred, every other to every third septum was
384 formed with cell separation in some aerial hyphae. In contrast, the average spore-type compartment
385 lengths were greater for the *ftsW* and *ftsI* mutants, 7.18 (± 8.32) μm and 5.73 (± 6.00) μm ,
386 respectively (Fig. S6), suggesting that in some hyphae with development-associated division
387 leading to cell separation, every sixth to seventh septum may have been completed all the way to
388 detachment of cells.

389 Phase-contrast microscopy showed that the divisome mutants were capable of division leading to
390 cell separation, but did not provide detail on the septum morphology when division failed. Of the
391 five isolated single mutants, *ftsL* and *ftsI* single mutants were selected for observation by electron
392 microscopy as representative examples of mutants affecting the putative FtsQ-DivIC-FtsL
393 complex and the FtsW-FtsI complex. TEM analysis for an *ftsL*-null mutant indicated that the

394 evenly-spaced constrictions in aerial hyphae observed by light microscopy represented complete
395 invaginations with very thick peptidoglycan (Fig. 1c), while *ftsI*-null mutants produced more
396 normal looking septa (Fig. 1c), but at a lower frequency.

397 While extremely rare in the wild type strain, branching within nascent spore chains was commonly
398 observed for the *ftsQ*, *divIC*, *ftsL*, *ftsW* and *ftsI* mutants. Both the cell division defect and the
399 observed branching phenotype in aerial hyphae were rescued in genetic complementation studies,
400 confirming that the phenotypes are associated with the deletion of these genes and not the result
401 of unlinked mutations (Fig. 1b).

402

403 **Core divisome proteins are not absolutely required for genome segregation in *S. venezuelae***

404 To further characterize the cell division and sporulation defects in *ftsZ*, *ftsZ2p*, *ftsQ*, *ftsL*, *divIC*,
405 *ftsW* and *ftsI* mutants of *S. venezuelae*, the cell wall was stained using WGA-FITC and nucleoids
406 were stained by propidium iodide (Figs. 3 and S7). Cell wall staining confirmed that hyphae of the
407 *ftsZ*-null mutant had no signs of invagination, vegetative cross walls or sporulation septa. In
408 addition, there was no evidence of DNA condensation either (Fig. 3). In contrast, partial nucleoid
409 condensation and segregation was observed for the *ftsZ2p* developmental promoter mutant (Fig.
410 3).

411 Consistent with observations from phase-contrast and TEM microscopy, *ftsQ*, *ftsL*, *divIC*, *ftsW*
412 and *ftsI* mutants showed very similar patterns of cell wall and DNA staining (Figs. 3 and S7). In
413 aerial hyphae with constrictions visible by light microscopy, ladders of nascent septal wall material
414 could be seen. However, these ladders were often not as regular as the evenly-spaced ones seen
415 for the wild type. DNA segregation was not grossly affected by the loss of any of these division
416 genes, but often was less uniform than for the wild type. Overall, under the laboratory conditions
417 that we tested, we conclude that *S. venezuelae* is able to lay down cell division septa and segregate
418 their genomes even in the absence of the core divisome genes *ftsQ*, *ftsL*, *divIC*, *ftsW* or *ftsI*. Future
419 avenues of research will be necessary to understand why these genes are conserved, yet their
420 products are not essential for septum formation for this filamentous bacterium.

421

422 **Assembly of FtsZ into ladder-like arrays of Z rings in sporogenic hyphae does not require**
423 **the core divisome genes *ftsQ*, *ftsL*, *divIC*, and *ftsW***

424 Next, using a subset of the mutants, we investigated to what extent the core divisome mutations
425 affected the localization and dynamics of FtsZ rings in *S. venezuelae*. In order to do this, *ftsZ-ypet*
426 (pKF351) was introduced into the Φ BT1 *att* site of *ftsQ*, *ftsL*, *divIC*, and *ftsW* mutants, as well as
427 the wild type strain, leading to production of FtsZ fused to the yellow fluorescent protein YPet in
428 addition to the native FtsZ. In vegetative hyphae sampled from standard liquid medium cultures at
429 different stages along the growth curve, we observed an apparently normal distribution of Z rings
430 in vegetative hyphae (not shown, but see also microfluidics data below). Further, sporulating
431 hyphae with multiple, closely and regularly-spaced Z rings were observed in both the wild type
432 strain and the *ftsQ*, *ftsL*, *divIC*, and *ftsW* mutants (Fig. 4), albeit examples of sporulating hyphae
433 were observed at a lower frequency in the mutants than in the wild type.

434 In order to observe more clearly how mutations in core divisome genes affect FtsZ dynamics and
435 Z-ring formation, we used microfluidics-based fluorescence live cell imaging, as described
436 previously [35, 47]. Representative micrographs of FtsZ ladders formed in the wild type and *ftsQ*
437 and *ftsW* mutants under these conditions are shown in Fig. 5. Time lapse images were also acquired
438 to visualize the FtsZ dynamics. In the wild type, during vegetative stage, typical Z rings are
439 observed that are highly dynamic as shown by their movement along the hyphae before they
440 stabilize at fixed positions and then increase in fluorescence intensity (Movie S1). Presumably,
441 these observed intense Z rings mark sites of vegetative cross-wall formation. A very similar pattern
442 was seen for formation of Z rings in early growth timepoints for vegetative hyphae of the *ftsQ* and
443 *ftsW* mutants (Movies S2 and S3, respectively). FtsZ dynamics were also visualized during the
444 sporulation stage for the wild type parent, where examples of the assembly of evenly-spaced Z
445 rings in ladder-like patterns could be seen in sporogenic hyphae (Movie S1, >10 hours of growth
446 in this sample). Intriguingly, similar development-associated FtsZ dynamics were observed in the
447 mutants for *ftsQ* (Movie S2) and *ftsW* (Movie S3) and the assembly of evenly-spaced FtsZ ladders
448 occurred. Closer inspection of the stability FtsZ ladder persistence was accomplished by
449 constructing a montage from timepoint images for the wild type parent and the *ftsQ* and *ftsW*
450 mutants (Fig. 6). The FtsZ ladders persist for approximately two hours for the wild type, but the
451 ladders do not show the same dynamics for the mutants. Certain FtsZ rings are lost over time in

452 the mutants, with the rungs of FtsZ ladders in the *ftsW* mutant being the least stable. These relative
453 FtsZ ladder stabilities correlate with the average lengths of mature spores that can be harvested
454 from surface grown cultures (Fig. S6), with the spores for *ftsQ* and *ftsW* mutants being
455 approximately 2X and 7X the length of those produced by the wild type.

456

457 **Physiological relevance of cumulative results**

458 Overall, the results clearly show that the products of the divisome genes *ftsQ*, *ftsL*, *divIC*, and *ftsW*
459 are not required for Z-ring assembly, for the single Z rings that are formed in vegetative hyphae
460 (normally leading to hyphal cross-walls in the wild type), and in sporogenic hyphae, where ladders
461 of regularly spaced Z rings are typically formed as part of sporulation septation. . In some
462 instances, Z-ring formation appeared essentially normal in the *ftsQ*, *ftsL*, *divIC*, and *ftsW* mutants.
463 The results are consistent with the previously observed ability of corresponding mutants in *S.*
464 *coelicolor* to form cross-walls and septa, at least under certain conditions [28-31]. It has been
465 speculated previously that *ftsW* may be required for Z-ring assembly, and may provide a membrane
466 attachment for FtsZ in both *S. coelicolor* and *Mycobacterium smegmatis* [31, 52]. Our results
467 presented here for *S. venezuelae* show that *ftsW* is not required for Z-ring formation.

468 The fact that core cell division proteins FtsQ, FtsL, DivIC, FtsW and FtsI are not strictly required
469 for cell division in *Streptomyces* spp. gives rise to interesting questions to be investigated in future
470 studies. For example, how is it possible to carry out cell division in the absence of the FtsQ-FtsL-
471 DivIC complex? Either the divisome in *Streptomyces* spp. can be stable and functional without
472 these proteins, or there are other proteins that can replace or reinforce these core divisome proteins.
473 In the latter case, such proteins would be pertinent to identify. Interestingly, co-
474 immunoprecipitation experiments identified 63 FtsQ-interacting proteins for *Mycobacterium*
475 *tuberculosis* and may point to homologs for further investigation [53].

476 Similarly, how can cell division occur in the absence of FtsW and its cognate transpeptidase FtsI?
477 The transpeptidase of FtsI has been shown to be nonessential for some other gram-positive
478 bacteria, although the protein is still physically required [54-56]. In the absence of FtsI, perhaps
479 FtsW functions with one or more of the many PBPs encoded for *S. venezuelae*. FtsI transpeptidase
480 activity can be supplied by other PBPs in *B. subtilis* [56]. FtsW co-purifies with two different PBPs

481 in a potential trimeric complex in *E. coli* [57]. FtsW has recently been identified as a peptidoglycan
482 transglycosylase (essentially a peptidoglycan polymerase) [27], in similarity to related RodA
483 SEDS proteins [58-60]. These are essential activities for formation of a cell division septum, and
484 the results presented here suggests that another peptidoglycan polymerase likely is recruited to Z
485 rings at division sites in order for the *S. venezuelae ftsW* mutant to form septa. As one possibility,
486 perhaps transglycosylase activity can be provided by an autonomous bifunctional class A PBP and
487 not by a SEDS protein. In support of that notion, evidence for intimate participation of bifunctional
488 PBPs in septum peptidoglycan synthesis has been accumulating [61]. Recent evidence suggests
489 that pneumococcal peptidoglycan is synthesized, in part, by bifunctional PBPs [62]. As another
490 possibility, one of the other three SEDS proteins encoded by streptomycete genomes [29, 31] may
491 be active either at the same time as FtsW and/or induced in the absence. It will be interesting to
492 see which protein(s) functions in a *ftsW* mutant and how it would be recruited to the divisome. Of
493 final note, we also have constructed strains individually expressing EFGP fusions to each protein
494 of the FtsQ-FtsL-DivIC or FtsI-FtsW complexes and the fluorescent localization signals are not
495 strong enough to publish (data not shown). Potentially, the weak fluorescence signal is indicative
496 of a low intracellular concentration. Again, future work will have to be done to learn if small
497 amounts of the proteins are needed for normal function, if another protein can substitute, or if
498 multiple SEDS-PBP pairs work simultaneously during sporulation septum formation.

499 **SUMMARY AND CONCLUSIONS**

500 In this study, we have established the contributions of known central cell division proteins in the
501 coordinated process of sporulation septation in *S. venezuelae*. Knowing the null phenotypes for
502 mutants lacking known players in cell division will be essential for future studies as we continue
503 to peel back the novel lineage-specific layers of controls evolved to govern the concerted
504 development-associated control of essential cell biological processes in streptomycetes.

505 In this study, we have taken advantage of the benefits of *S. venezuelae* to visualize the synchronous
506 events being orchestrated within sporogenic hyphae by live-cell time-lapse microscopy because
507 this species undergoes differentiation under submerged growth conditions. The data show that
508 ladder-like assemblages of evenly-spaced FtsZ rings typically form in all of the characterized core
509 divisome mutants. Thus, the tested divisome components are not required for that early
510 coordinated event. However, once formed the Z-rings appear to be unstable and a number of rings

511 prematurely disband. The loss of coordination results in irregular spacing between completed septa
512 and irregular spore size, as seen in the divisome mutants.

513 Evidence has accumulated for subcomplex formation of FtsQ-FtsL-DivIC and FtsW-FtsI before
514 participation in the divisome. For *S. venezuelae*, combining mutations of genes encoding these
515 components do not result in synthetic phenotypes. The result is consistent with the interpretation
516 that the loss of any one component disrupts the function of the subcomplex. While the
517 subcomplexes are not absolutely required, they do contribute to the stability of the synchronous
518 tandem arrays of divisome complexes as visualized by FtsZ-YPet. Recently, analysis of bacterial
519 dynamins DynA and DynB for *S. venezuelae* showed that they interact with the division machinery
520 [18], contribute to Z-ring stability and mutants encoding those proteins have somewhat similar
521 phenotypes as the divisome mutants reported here. Future work will be needed to understand how
522 these components interact and are regulated to synchronously coordinate sporulation septum
523 formation.

524

525 **ACKNOWLEDGEMENTS**

526 SC and JRM thank John Stolz for help with electron microscopy and John Pollock and Philip
527 Auron for help with fluorescence microscopy.

528

529 **Funding information**

530 Support for this work was provided by National Institutes of Health grant GM096268 (JRM),
531 Swedish Research Council grant 621-2010-4463 (KF), Crafoord Foundation grant 20140962
532 (KF) and Wenner-Gren guest researcher grant (KF and JRM); stipend support was provided by
533 the Bayer School of Natural and Environmental Sciences, Duquesne University (SC); BCS was
534 supported by the Sven och Lilly Lawskis fond för naturvetenskaplig forskning, Stiftelsen Jörgen
535 Lindströms stipendiefond, and the Royal Physiographic Society of Lund.

536

537 **Conflicts of interest**

538 The authors declare no conflict of interests.

539 REFERENCES

- 540 1. **Hopwood DA.** Forty years of genetics with *Streptomyces*: from *in vivo* through
541 *in vitro* to *in silico*. *Microbiology*. 1999;145:2183-202. Doi: 10.1099/00221287-145-9-
542 2183
- 543 2. **Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Meier-**
544 **Kolthoff JP, et al.** Taxonomy, physiology, and natural products of Actinobacteria.
545 *Microbiol Mol Biol Rev*. 2016;80:1-43. Doi: 10.1128/MMBR.00019-15
- 546 3. **Bush MJ, Tschowri N, Schlimpert S, Flårdh K, Buttner MJ.** c-di-GMP
547 signalling and the regulation of developmental transitions in streptomycetes. *Nat Rev*
548 *Microbiol*. 2015;13:749-60. Doi: 10.1038/nrmicro3546
- 549 4. **McCormick JR, Flårdh K.** Signals and regulators that govern *Streptomyces*
550 development. *FEMS Microbiol Rev*. 2012;36:206-31. Doi:
551 5. **Elliot MA, Flårdh K.** Streptomycete spores. eLS. Chichester: John Wiley &
552 Sons Ltd; 2020. Doi: 10.1002/9780470015902.a0000308.pub3
- 553 6. **Jakimowicz D, van Wezel GP.** Cell division and DNA segregation in
554 *Streptomyces*: how to build a septum in the middle of nowhere? *Mol Microbiol*.
555 2012;85:393-404. Doi: 10.1111/j.1365-2958.2012.08107.x
- 556 7. **McCormick JR.** Cell division is dispensable but not irrelevant in *Streptomyces*.
557 *Curr Opin Microbiol*. 2009;12:689-98. Doi:
558 8. **McCormick JR, Su EP, Driks A, Losick R.** Growth and viability of
559 *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. *Mol Microbiol*.
560 1994;14:243-54. Doi:
561 9. **Santos-Beneit F, Roberts DM, Cantlay S, McCormick JR, Errington J.**
562 A mechanism for FtsZ-independent proliferation in *Streptomyces*. *Nat Commun*.
563 2017;8:1378. Doi: 10.1038/s41467-017-01596-z
- 564 10. **Wildermuth H, Hopwood DA.** Septation during sporulation in *Streptomyces*
565 *coelicolor*. *J Gen Microbiol*. 1970;60:57-9. Doi:
566 11. **Flårdh K, Leibovitz E, Buttner MJ, Chater KF.** Generation of a non-
567 sporulating strain of *Streptomyces coelicolor* A3(2) by the manipulation of a
568 developmentally controlled *ftsZ* promoter. *Mol Microbiol*. 2000;38:737-49. Doi:
569 12. **den Hengst CD, Tran NT, Bibb MJ, Chandra G, Leskiw BK, Buttner**
570 **MJ.** Genes essential for morphological development and antibiotic production in
571 *Streptomyces coelicolor* are targets of BldD during vegetative growth. *Mol Microbiol*.
572 2010;78:361-79. Doi: 10.1111/j.1365-2958.2010.07338.x
- 573 13. **Tschowri N, Schumacher MA, Schlimpert S, Chinnam NB, Findlay KC,**
574 **Brennan RG, et al.** Tetrameric c-di-GMP mediates effective transcription factor
575 dimerization to control *Streptomyces* development. *Cell*. 2014;158:1136-47. Doi:
576 10.1016/j.cell.2014.07.022
- 577 14. **Bush MJ, Bibb MJ, Chandra G, Findlay KC, Buttner MJ.** Genes required
578 for aerial growth, cell division, and chromosome segregation are targets of WhiA before
579 sporulation in *Streptomyces venezuelae*. *MBio*. 2013;4:e00684-13. Doi:
580 10.1128/mBio.00684-13
- 581 15. **Bush MJ, Chandra G, Bibb MJ, Findlay KC, Buttner MJ.** Genome-wide
582 chromatin immunoprecipitation sequencing analysis shows that WhiB is a transcription
583 factor that cocontrols its regulon with WhiA to initiate developmental cell division in
584 *Streptomyces*. *MBio*. 2016;7:e00523-16. Doi: 10.1128/mBio.00523-16

- 585 16. **Al-Bassam MM, Bibb MJ, Bush MJ, Chandra G, Buttner MJ.** Response
586 regulator heterodimer formation controls a key stage in *Streptomyces* development.
587 *PLoS Genet.* 2014;10:e1004554. Doi: 10.1371/journal.pgen.1004554
- 588 17. **Willemse J, Borst JW, de Waal E, Bisseling T, van Wezel GP.** Positive
589 control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces*.
590 *Genes Dev.* 2011;25:89-99. Doi:
591 18. **Schlimpert S, Wasserstrom S, Chandra G, Bibb MJ, Findlay KC,**
592 **Flårdh K, et al.** Two dynamin-like proteins stabilize FtsZ rings during *Streptomyces*
593 sporulation. *Proc Natl Acad Sci USA.* 2017;114:E6176-E83. Doi:
594 10.1073/pnas.1704612114
- 595 19. **Du S, Lutkenhaus J.** Assembly and activation of the *Escherichia coli* divisome.
596 *Mol Microbiol.* 2017;105:177-87. Doi: 10.1111/mmi.13696
- 597 20. **Haeusser DP, Margolin W.** Splitsville: structural and functional insights into
598 the dynamic bacterial Z ring. *Nat Rev Microbiol.* 2016;14:305-19. Doi:
599 10.1038/nrmicro.2016.26
- 600 21. **Ortiz C, Natale P, Cueto L, Vicente M.** The keepers of the ring: regulators of
601 FtsZ assembly. *FEMS Microbiol Rev.* 2016;40:57-67. Doi: 10.1093/femsre/fuv040
- 602 22. **Duman R, Ishikawa S, Celik I, Strahl H, Ogasawara N, Troc P, et al.**
603 Structural and genetic analyses reveal the protein SepF as a new membrane anchor for
604 the Z ring. *Proc Natl Acad Sci USA.* 2013;110:E4601-E10. Doi:
605 10.1073/pnas.1313978110
- 606 23. **Zhang L, Willemse J, Claessen D, van Wezel GP.** SepG coordinates
607 sporulation-specific cell division and nucleoid organization in *Streptomyces coelicolor*.
608 *Open Biol.* 2016;6:150164. Doi: 10.1098/rsob.150164
- 609 24. **Del Sol R, Mullins JG, Grantcharova N, Flårdh K, Dyson P.** Influence of
610 CrgA on assembly of the cell division protein FtsZ during development of *Streptomyces*
611 *coelicolor*. *J Bacteriol.* 2006;188:1540-50. Doi:
612 25. **Buddelmeijer N, Beckwith J.** A complex of the *Escherichia coli* cell division
613 proteins FtsL, FtsB and FtsQ forms independently of its localization to the septal region.
614 *Mol Microbiol.* 2004;52:1315-27. Doi: 10.1111/j.1365-2958.2004.04044.x
- 615 26. **Boes A, Olatunji S, Breukink E, Terrak M.** Regulation of the peptidoglycan
616 polymerase activity of PBP1b by antagonist actions of the core divisome proteins FtsBLQ
617 and FtsN. *mBio.* 2019;10:e01912-18. Doi: 10.1128/mBio.01912-18
- 618 27. **Taguchi A, Welsh MA, Marmont LS, Lee W, Sjodt M, Kruse AC, et al.**
619 FtsW is a peptidoglycan polymerase that is functional only in complex with its cognate
620 penicillin-binding protein. *Nat Microbiol.* 2019;4:587-94. Doi: 10.1038/s41564-018-
621 0345-x
- 622 28. **Bennett JA, Aimino RM, McCormick JR.** *Streptomyces coelicolor* genes
623 *ftsL* and *divIC* play a role in cell division but are dispensable for colony formation. *J*
624 *Bacteriol.* 2007;189:8982-92. Doi:
625 29. **Bennett JA, Yarnall J, Cadwallader AB, Kuennen R, Bidey P,**
626 **Stadelmaier B, et al.** Medium-dependent phenotypes of *Streptomyces coelicolor* with
627 mutations in *ftsI* or *ftsW*. *J Bacteriol.* 2009;191:661-4. Doi:
628 30. **McCormick JR, Losick R.** Cell division gene *ftsQ* is required for efficient
629 sporulation but not growth and viability in *Streptomyces coelicolor* A3(2). *J Bacteriol.*
630 1996;178:5295-301. Doi:

- 631 31. **Mistry BV, Del Sol R, Wright C, Findlay K, Dyson P.** FtsW is a
632 dispensable cell division protein required for Z-ring stabilization during sporulation
633 septation in *Streptomyces coelicolor*. *J Bacteriol.* 2008;190:5555-66. Doi:
634 10.1128/JB.00398-08
- 635 32. **Ausmees N, Wahlstedt H, Bagchi S, Elliot MA, Buttner MJ, Flardh K.**
636 SmeA, a small membrane protein with multiple functions in *Streptomyces* sporulation
637 including targeting of a SpoIIIE/FtsK-like protein to cell division septa. *Mol Microbiol.*
638 2007;65:1458-73. Doi: 10.1111/j.1365-2958.2007.05877.x
- 639 33. **Dedrick RM, Wildschutte H, McCormick JR.** Genetic interactions of *smc*,
640 *ftsK*, and *parB* genes in *Streptomyces coelicolor* and their developmental genome
641 segregation phenotypes. *J Bacteriol.* 2009;191:320-32. Doi:
642 34. **Wang L, Yu Y, He X, Zhou X, Deng Z, Chater KF, et al.** Role of an FtsK-
643 like protein in genetic stability in *Streptomyces coelicolor* A3(2). *J Bacteriol.*
644 2007;189:2310-8. Doi:
645 35. **Schlimpert S, Flårdh K, Buttner M.** Fluorescence time-lapse imaging of the
646 complete *S. venezuelae* life cycle using a microfluidic device. *J Vis Exp.* 2016:e53863.
647 Doi: 10.3791/53863
- 648 36. **Stuttard C.** Temperate phages of *Streptomyces venezuelae*: lysogeny and
649 specificity shown by phages SV1 and SV2. *Microbiology.* 1982;128:115-21. Doi:
650 37. **Bibb MJ, Domonkos A, Chandra G, Buttner MJ.** Expression of the chaplin
651 and rodlin hydrophobic sheath proteins in *Streptomyces venezuelae* is controlled by
652 sigma(BldN) and a cognate anti-sigma factor, RsbN. *Mol Microbiol.* 2012;84:1033-49.
653 Doi: 10.1111/j.1365-2958.2012.08070.x
- 654 38. **Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA.** Practical
655 *Streptomyces* Genetics. Norwich, UK: The John Innes Foundation; 2000.
- 656 39. **Sambrook J, Fritsch EF, Maniatis T.** Molecular Cloning: A Laboratory
657 Manual. Second ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press;
658 1989.
- 659 40. **Datsenko KA, Wanner BL.** One-step inactivation of chromosomal genes in
660 *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A.* 2000;97:6640-5.
661 Doi: 10.1073/pnas.120163297
- 662 41. **Gust B, Challis GL, Fowler K, Kieser T, Chater KF.** PCR-targeted
663 *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of
664 the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci U S A.* 2003;100:1541-6. Doi:
665 10.1073/pnas.0337542100
- 666 42. **Datsenko KA, Wanner BW.** One-step inactivation of chromosomal genes in
667 *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA.* 2000;97:6640-5.
668 Doi: 10.1073/pnas.120163297
- 669 43. **Gust B, Challis GL, Fowler K, Kieser T, Chater KF.** PCR-targeted
670 *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of
671 the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci USA.* 2003;100:1541-6. Doi:
672 10.1073/pnas.0337542100
- 673 44. **Cherepanov PP, Wackernagel W.** Gene disruption in *Escherichia coli*: TcR
674 and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance
675 determinant. *Gene.* 1995;158:9-14. Doi: 10.1016/0378-1119(95)00193-a

- 676 45. **Gregory MA, Till R, Smith MC.** Integration site for *Streptomyces* phage
677 phiBT1 and development of site-specific integrating vectors. *J Bacteriol.*
678 2003;185:5320-3. Doi: 10.1128/jb.185.17.5320-5323.2003
- 679 46. **Donczew M, Mackiewicz P, Wrobel A, Flärdh K, Zakrzewska-**
680 **Czerwinska J, Jakimowicz D.** ParA and ParB coordinate chromosome segregation
681 with cell elongation and division during *Streptomyces* sporulation. *Open Biol.*
682 2016;6:150263. Doi: 10.1098/rsob.150263
- 683 47. **Sen BC, Wasserstrom S, Findlay KC, Söderholm N, Sandblad L, von**
684 **Wachenfeldt C, et al.** Specific amino acid substitutions in β strand S2 of FtsZ cause
685 spiraling septation and impair assembly cooperativity in *Streptomyces*. *Mol Microbiol.*
686 2019;112:184-98. Doi: 10.1111/mmi.14262
- 687 48. **Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M,**
688 **Pietzsch T, et al.** Fiji: an open-source platform for biological-image analysis. *Nat*
689 *Methods.* 2012;9:676-82. Doi: 10.1038/nmeth.2019
- 690 49. **Bush MJ, Chandra G, Al-Bassam MM, Findlay KC, Buttner MJ.** BldC
691 delays entry into development to produce a sustained period of vegetative growth in
692 *Streptomyces venezuelae*. *MBio.* 2019;10:e02812-18. Doi: 10.1128/mBio.02812-18
- 693 50. **Kwak J, Dharmatilake AJ, Jiang H, Kendrick KE.** Differential regulation
694 of *ftsZ* transcription during septation of *Streptomyces griseus*. *J Bacteriol.*
695 2001;183:5092-101. Doi:
696 51. **Trip EN, Scheffers DJ.** A 1 MDa protein complex containing critical
697 components of the *Escherichia coli* divisome. *Sci Rep.* 2015;5:18190. Doi:
698 10.1038/srep18190
- 699 52. **Datta P, Dasgupta A, Bhakta S, Basu J.** Interaction between FtsZ and FtsW
700 of *Mycobacterium tuberculosis*. *J Biol Chem.* 2002;277:24983-7. Doi:
701 10.1074/jbc.M203847200
- 702 53. **Wu KJ, Zhang J, Baranowski C, Leung V, Rego EH, Morita YS, et al.**
703 Characterization of conserved and novel septal factors in *Mycobacterium smegmatis*. *J*
704 *Bacteriol.* 2018;200:e00649-17. Doi: 10.1128/JB.00649-17
- 705 54. **Morales Angeles D, Liu Y, Hartman AM, Borisova M, Borges AD, de**
706 **Kok N, et al.** Pentapeptide-rich peptidoglycan at the *Bacillus subtilis* cell-division site.
707 *Mol Microbiol.* 2017;104:319-33. Doi: 10.1111/mmi.13629
- 708 55. **Peters K, Schweizer I, Beilharz K, Stahlmann C, Veening JW,**
709 **Hakenbeck R, et al.** *Streptococcus pneumoniae* PBP2x mid-cell localization requires
710 the C-terminal PASTA domains and is essential for cell shape maintenance. *Mol*
711 *Microbiol.* 2014;92:733-55. Doi: 10.1111/mmi.12588
- 712 56. **Sassine J, Xu MZ, Sidiq KR, Emmins R, Errington J, Daniel RA.**
713 Functional redundancy of division specific penicillin-binding proteins in *Bacillus*
714 *subtilis*. *Mol Microbiol.* 2017;106:304-18. Doi: 10.1111/mmi.13765
- 715 57. **Leclercq S, Derouaux A, Olatunji S, Fraipont C, Egan AJ, Vollmer W,**
716 **et al.** Interplay between Penicillin-binding proteins and SEDS proteins promotes
717 bacterial cell wall synthesis. *Sci Rep.* 2017;7:43306. Doi: 10.1038/srep43306
- 718 58. **Cho H, Wivagg CN, Kapoor M, Barry Z, Rohs PDA, Suh H, et al.**
719 Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families
720 functioning semi-autonomously. *Nat Microbiol.* 2016;1:16172. Doi:
721 10.1038/nmicrobiol.2016.172

- 722 59. **Emami K, Guyet A, Kawai Y, Devi J, Wu LJ, Allenby N, et al.** RodA as
723 the missing glycosyltransferase in *Bacillus subtilis* and antibiotic discovery for the
724 peptidoglycan polymerase pathway. *Nat Microbiol.* 2017;2:16253. Doi:
725 10.1038/nmicrobiol.2016.253
- 726 60. **Meeske AJ, Riley EP, Robins WP, Uehara T, Mekalanos JJ, Kahne D,**
727 **et al.** SEDS proteins are a widespread family of bacterial cell wall polymerases. *Nature.*
728 2016;537:634-8. Doi: 10.1038/nature19331
- 729 61. **Pazos M, Peters K, Casanova M, Palacios P, VanNieuwenhze M,**
730 **Breukink E, et al.** Z-ring membrane anchors associate with cell wall synthases to
731 initiate bacterial cell division. *Nat Commun.* 2018;9:5090. Doi: 10.1038/s41467-018-
732 07559-2
- 733 62. **Straume D, Piechowiak KW, Olsen S, Stamsas GA, Berg KH, Kjos M,**
734 **et al.** Class A PBPs have a distinct and unique role in the construction of the
735 pneumococcal cell wall. *Proc Natl Acad Sci USA.* 2020;117:6129-38. Doi:
736 10.1073/pnas.1917820117

737

738 **FIGURE LEGENDS**

739 **Figure 1. Construction and complementation of *S. venezuelae* strains mutant for core**
740 **division genes.**

741 **(a) A physical map of the *dcw* cluster in *S. venezuelae* and the genetic locus of *divIC*.** Maps
742 of two regions of the *S. venezuelae* chromosome are shown that contain genes encoding core
743 proteins of the divisome. In each of the two loci, all genes are in the same orientation as the
744 divisome genes. Regions replaced with an apramycin-resistance cassette or an unmarked in-
745 frame deletion mutation are shown above the maps. DNA fragments used for constructing
746 genetic complementation plasmids are shown below the map. **(b) Phase-contrast microscopy of**
747 **wild type and mutant phenotypes and mutant phenotypes following genetic**
748 **complementation.** All images are phase-contrast micrographs of cover slip impressions from
749 cultures grown for 2 days at 30°C on MYM agar. The top row contains wild type *S. venezuelae*
750 strain containing the empty complementation vector on the left (wt). Immediately adjacent are
751 shown seven division mutants containing the empty complementation vector pMS82. In the
752 bottom row are shown the seven division mutants containing a complementing fragment cloned
753 into pMS82 which restores sporulation to wild type levels. Scale bar, 5 µm. **(c) Transmission**
754 **electron micrographs reveal septation and cell wall defects in the *ftsZ*, *ftsI* and *ftsL* mutants.**
755 Cells were grown for 2 days at 30°C on MYM agar and thin sections were viewed by
756 transmission electron microscopy. Mainly spores were observed for the wild type strain (wt). No
757 examples of vegetative cross-walls and sporulation septa were observed for the *ftsZ*-null mutant.
758 White arrow heads indicate formed unresolved sporulation septa in aerial hyphae for the *ftsL* and
759 *ftsI* mutants. Scale bar, 500 nm.

760

761 **Figure 2. Double and triple divisome mutants do not have additive or synergistic division**
762 **phenotypes.**

763 The strains were grown for two days on MYM agar medium at 30°C. Shown are phase-contrast
764 images from impression coverslips of aerial hyphae for double and triple mutant strains. Aerial
765 hyphae of double and triple mutants frequently contain evenly-spaced constrictions as do the
766 single mutants. The double and triple mutant phenotypes are strikingly similar to the single
767 mutants (Fig. 1b) and do not result in synthetic division phenotypes. Scale bar, 5 µm.

768

769 **Figure 3. DNA segregation and cell wall phenotypes of *S. venezuelae* division mutants.**

770 Cells were grown for 2 days at 30°C on MYM agar and cover slips were pressed onto confluent
771 lawns. Samples of aerial hyphae were stained for cell wall (green) and DNA (red) and viewed by
772 epifluorescence microscopy. The top row contains corresponding DIC light images. Wild type
773 samples contained mainly spores and spore chains. Examples of aerial hyphae of mutant strains
774 \DeltaftsZ , $ftsZ\Delta 2p$, \DeltaftsL and \DeltaftsI are shown. Scale bar, 5 µm.

775

776 **Figure 4. Z-ring assembly in sporogenic hypha of *S. venezuelae* divisome mutants.**

777 Batch cultures were grown in a standard fashion in liquid MYM at 30°C and samples were fixed
778 by formaldehyde treatment before cells were mounted for microscopy. Representative
779 micrographs of sporulating hyphae with FtsZ ladders are shown, visualized using YPet-tagged
780 FtsZ. Shown are the wild type control strain and the indicated divisome mutants into which
781 plasmid pKF351[P_{ftsZ} - $ftsZ$ - $ypet$] had been introduced. Scale bars, 2 µm.

782

783 **Figure 5. Live-cell imaging of Z-ring assembly in sporogenic hypha of *S. venezuelae* wild**
784 **type and *ΔftsQ* and *ΔftsW* mutants.**

785 Cultures were grown in MYM at 30°C using a microfluidic system. Representative micrographs
786 of unfixed sporulating hyphae with FtsZ ladders are shown, visualized using YPet-tagged FtsZ.
787 Shown are the wild type control strain and the indicated divisome mutants into which plasmid
788 pKF351[*P_{ftsZ}-ftsZ-ypet*] had been introduced. Scale bars, 2 μm.

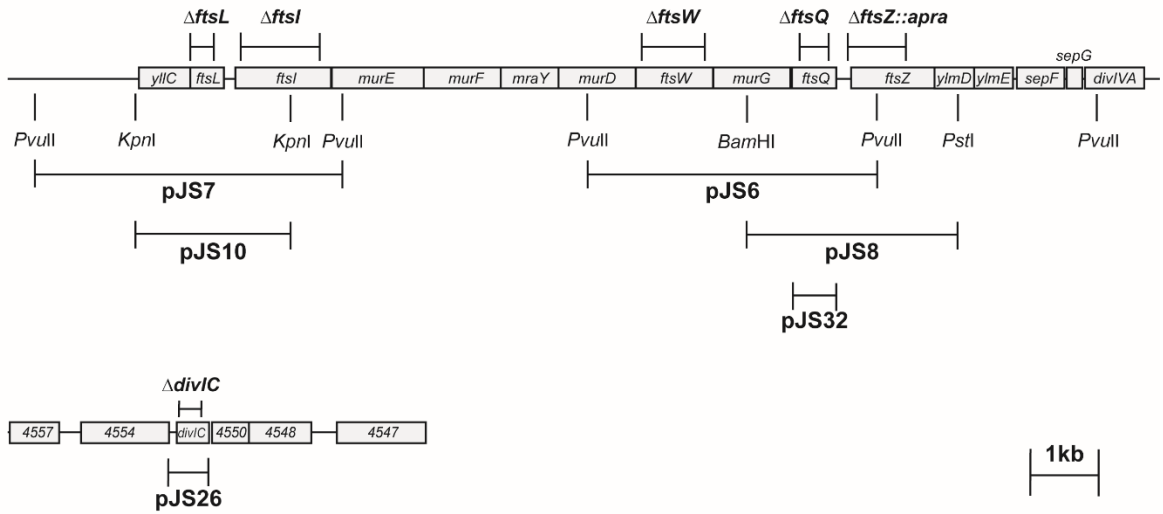
789

790 **Figure 6. FtsQ and FtsW stabilize Z-rings during sporulation-specific cell division.**

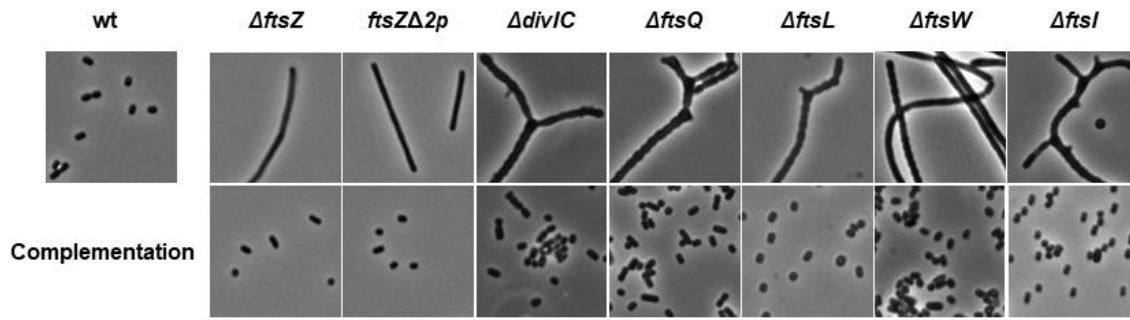
791 Shown is a montage of representative time series documenting FtsZ dynamics during spore
792 formation. Strains were grown in liquid MYM at 30°C using a microfluidic system. Fluorescence
793 images of FtsZ-YPet signal were obtained from time-lapse microscopy (top) and the
794 corresponding phase-contrast images are also shown (bottom). Shown are montages of the wild
795 type control strain and the indicated divisome mutants into which plasmid pKF351[*P_{ftsZ}-ftsZ-*
796 *ypet*] has been introduced. Time intervals between images were kept at 20 min. In addition, zero
797 min was considered as the time wherein the shown hypha had undergone arrest of tip extension
798 before sporulation septation began. Scale bars, 2 μm.

799

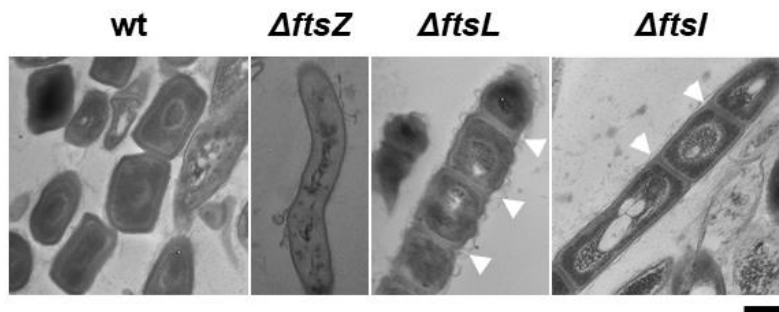
(a)



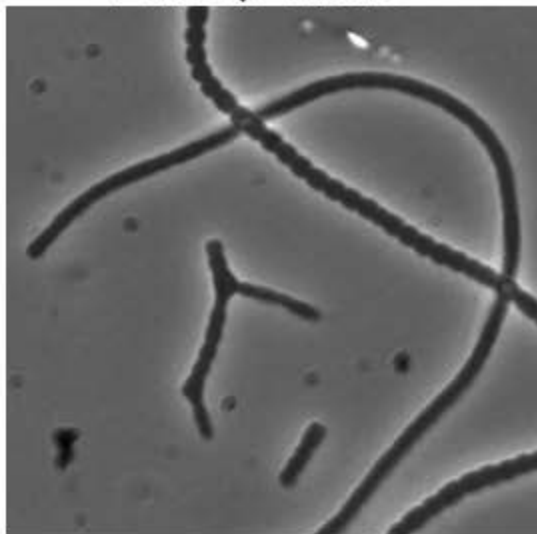
(b)



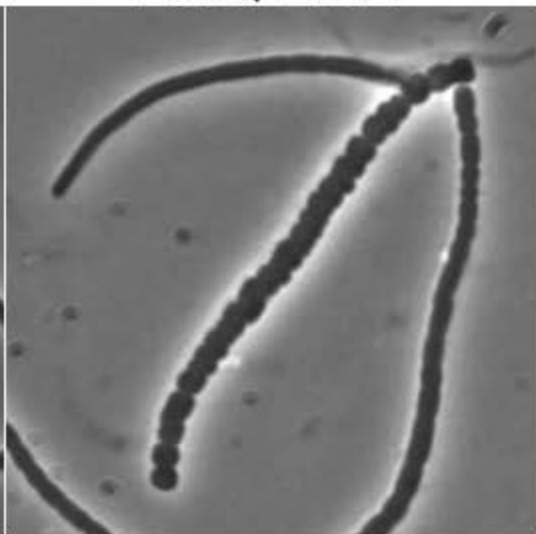
(c)



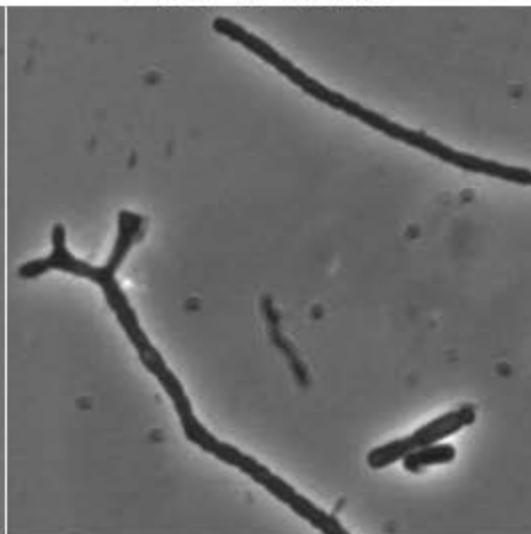
ΔftsQ ΔdivIC



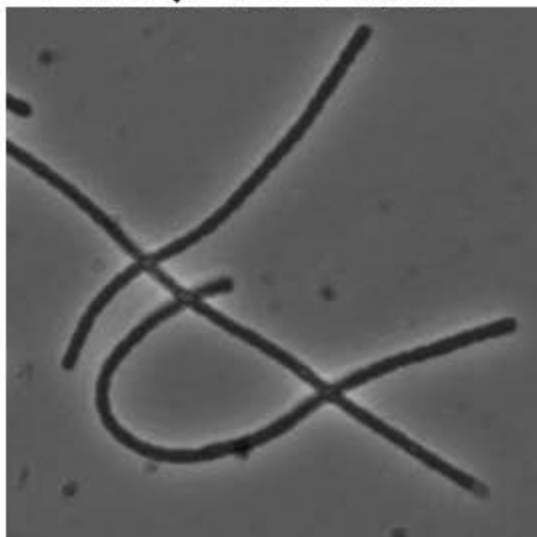
ΔftsQ ΔftsL



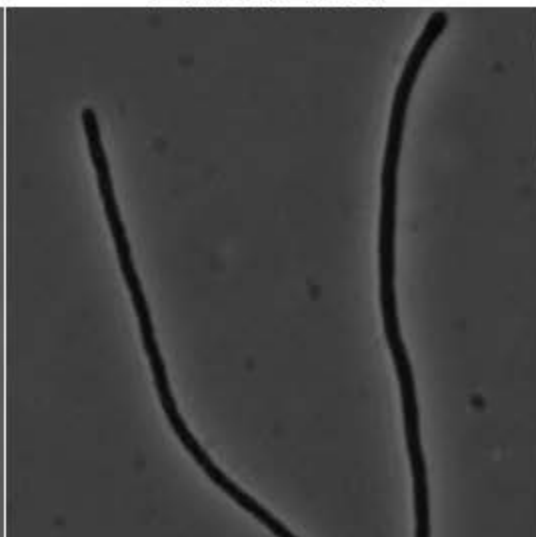
ΔftsL ΔdivIC



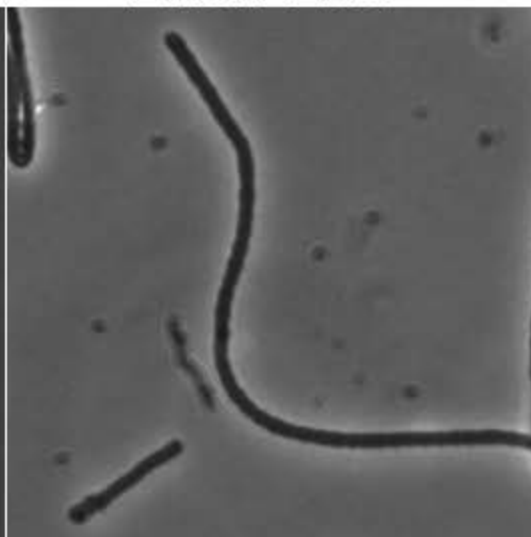
ΔftsQ ΔftsL ΔdivIC

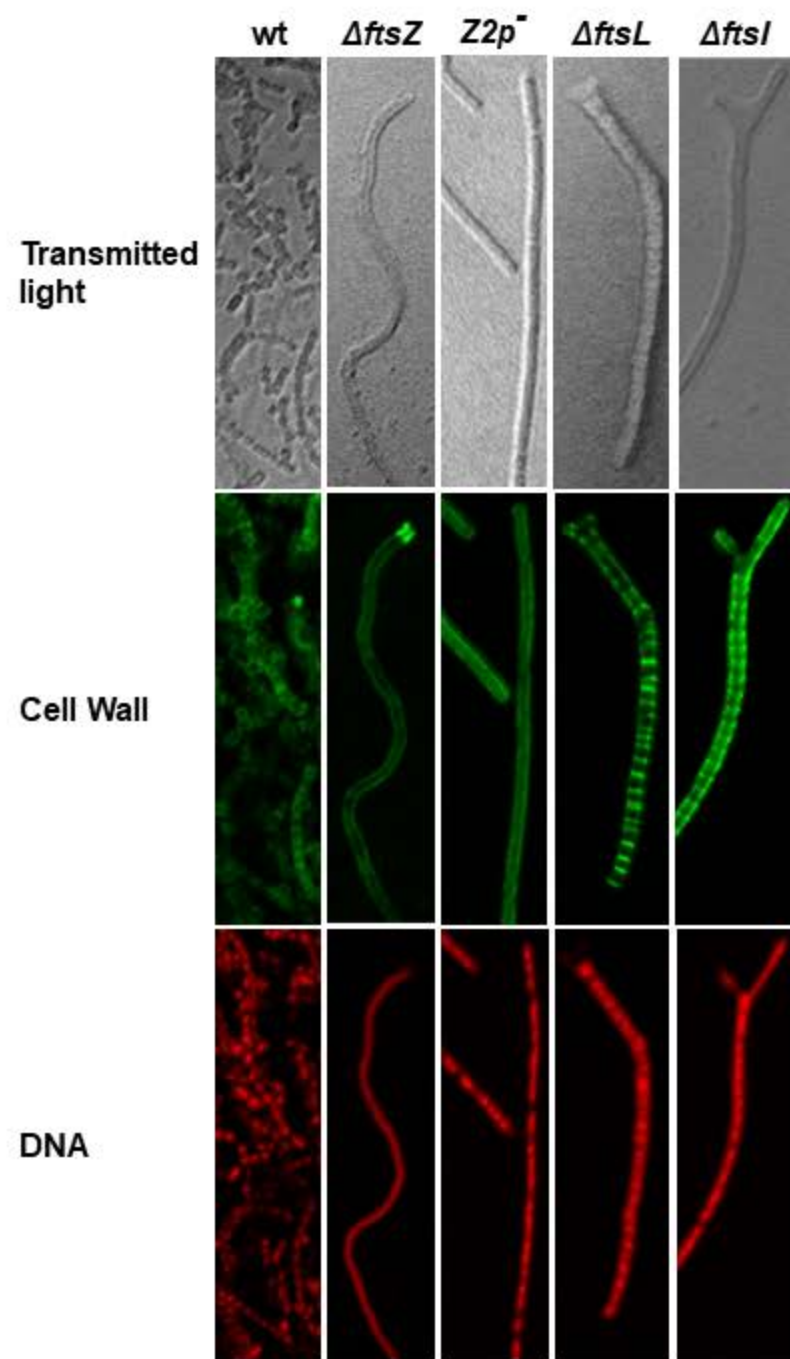


ΔftsL ΔftsI



ΔftsW ΔftsI

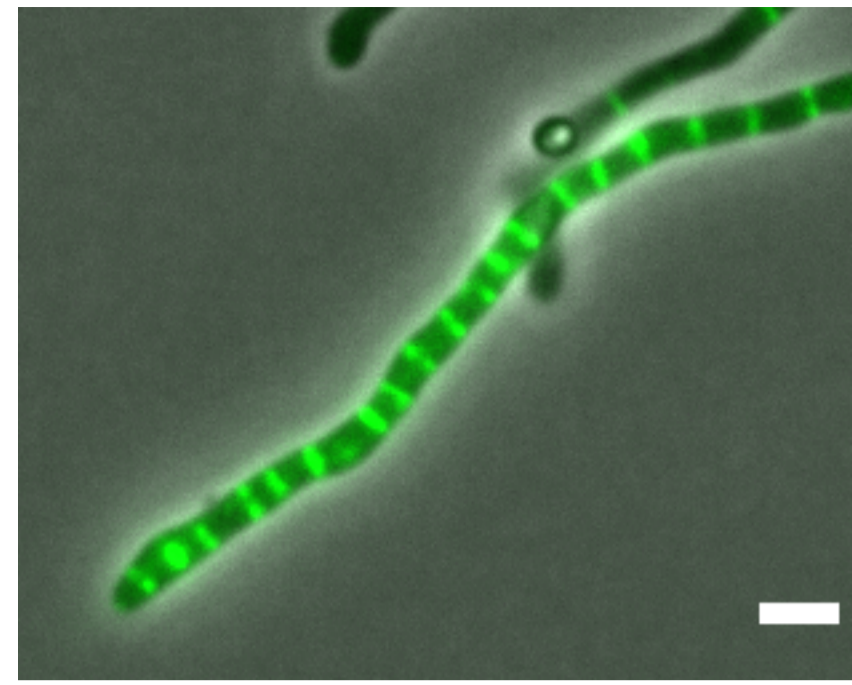
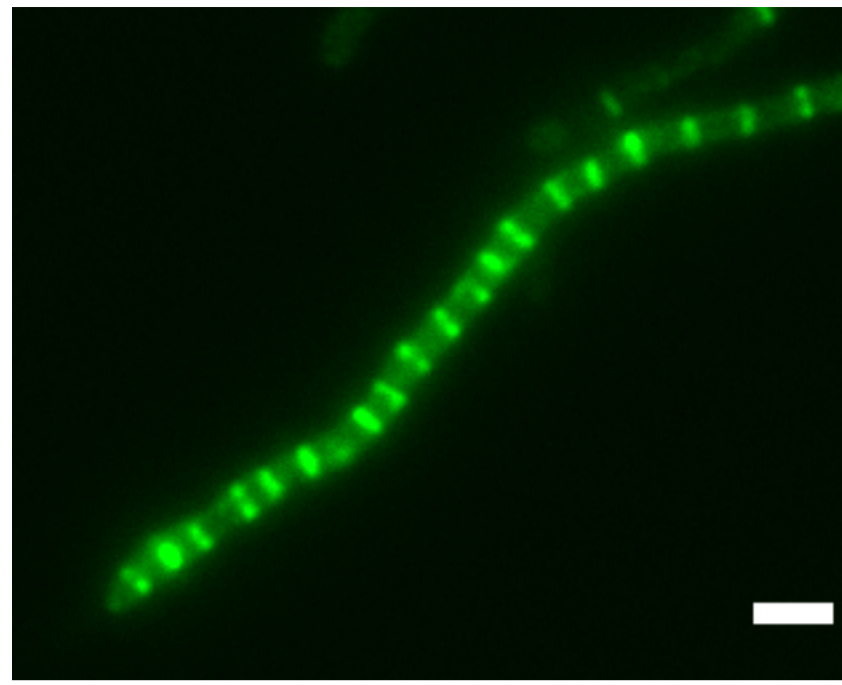
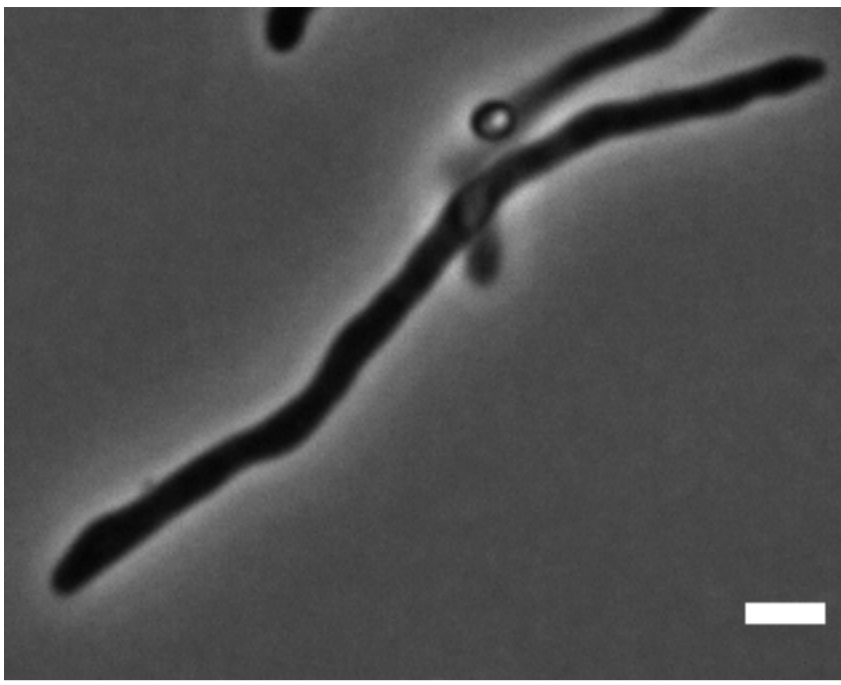
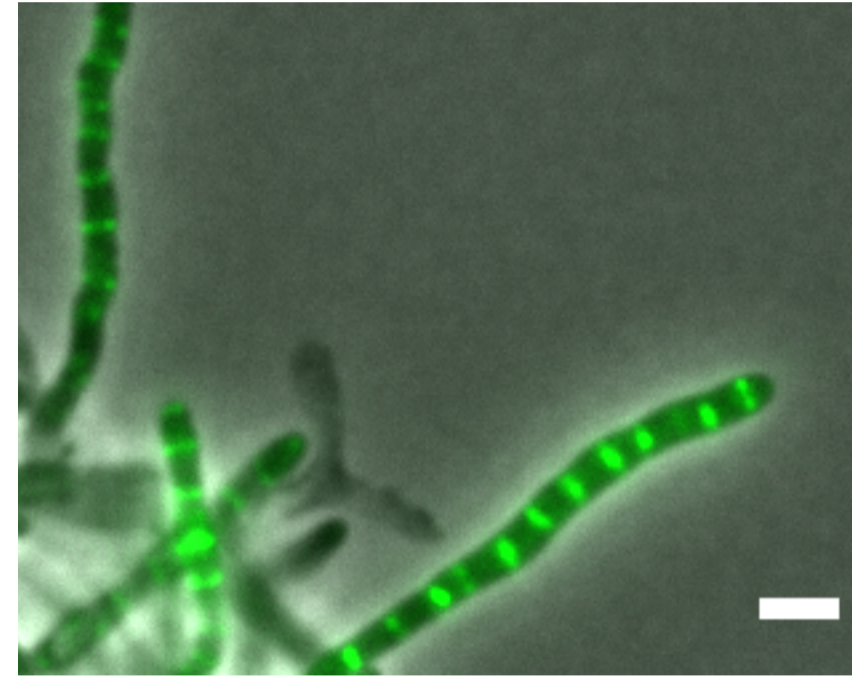
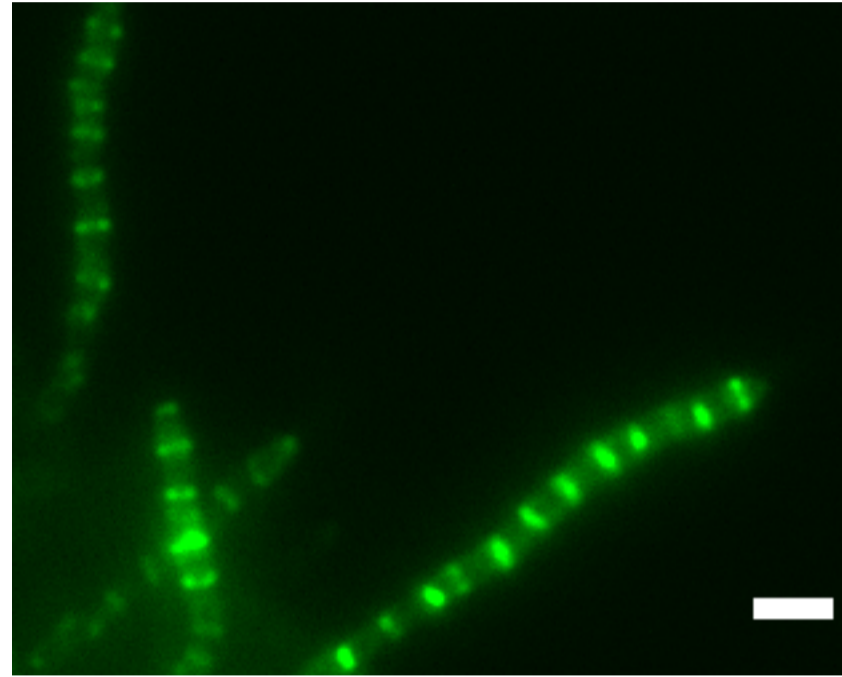
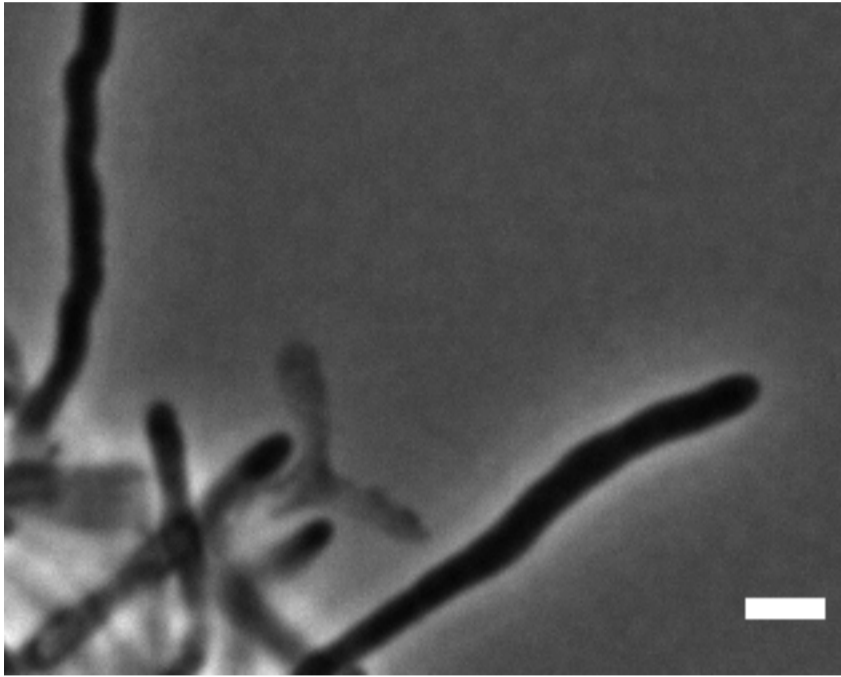
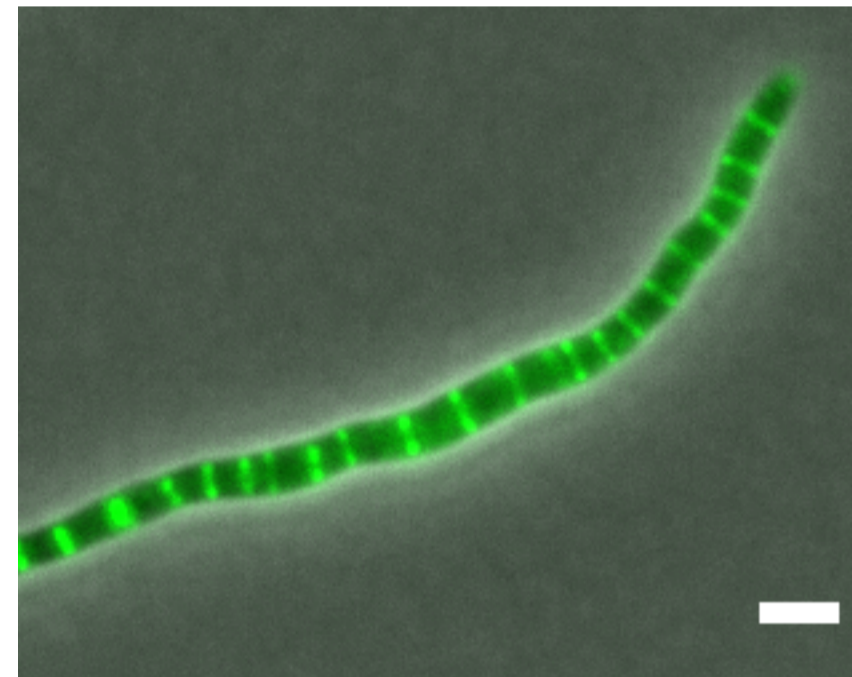
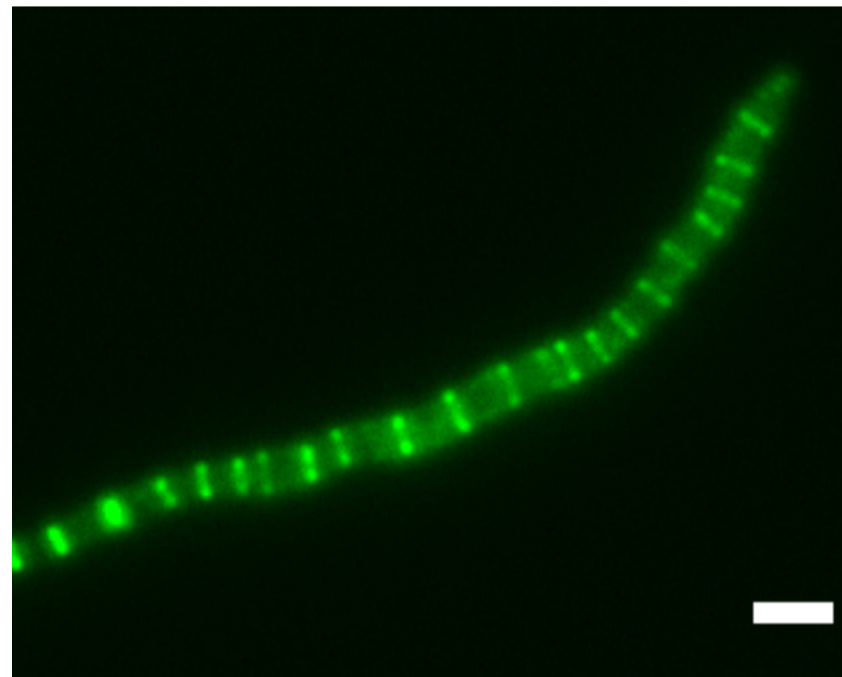
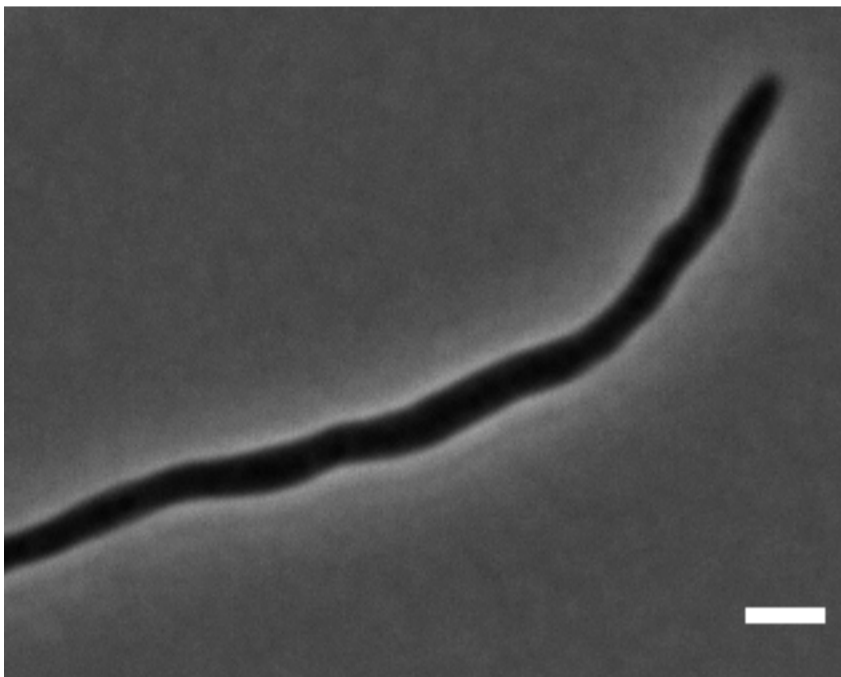
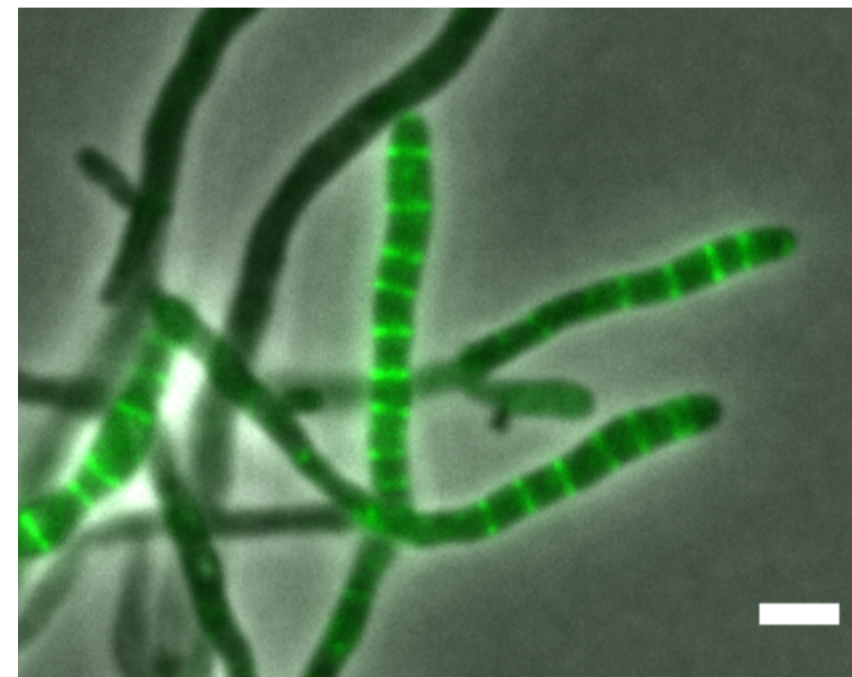
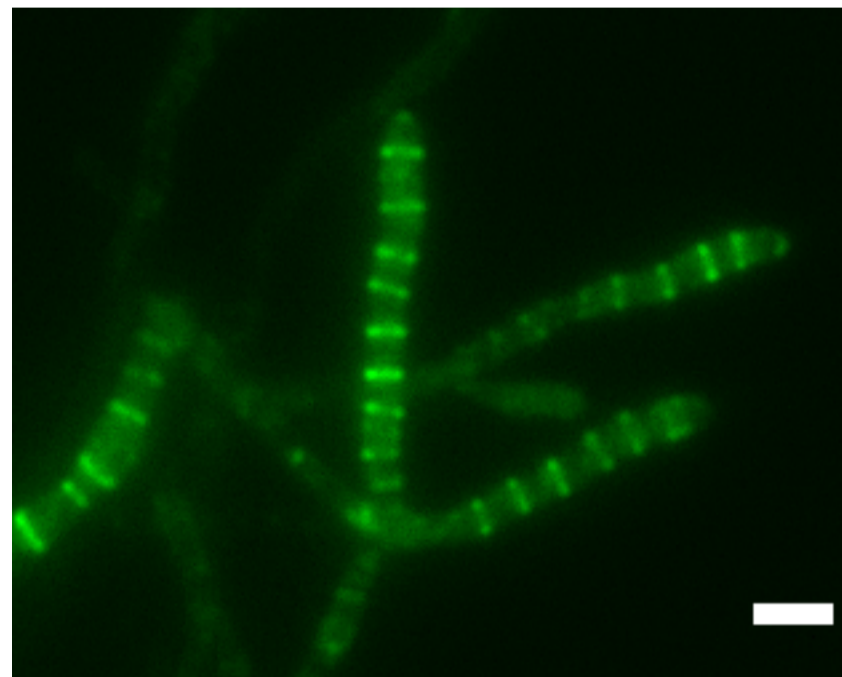
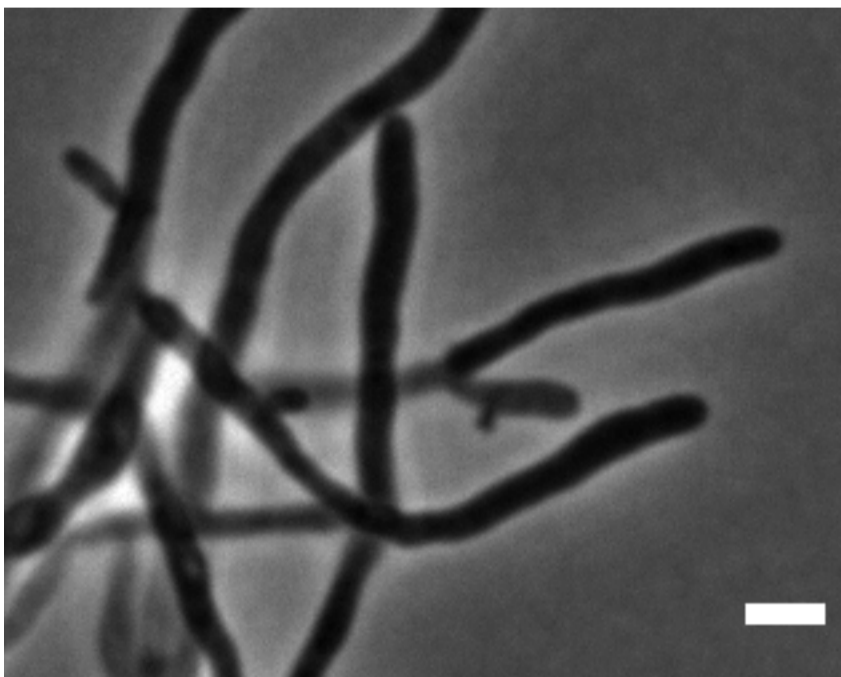
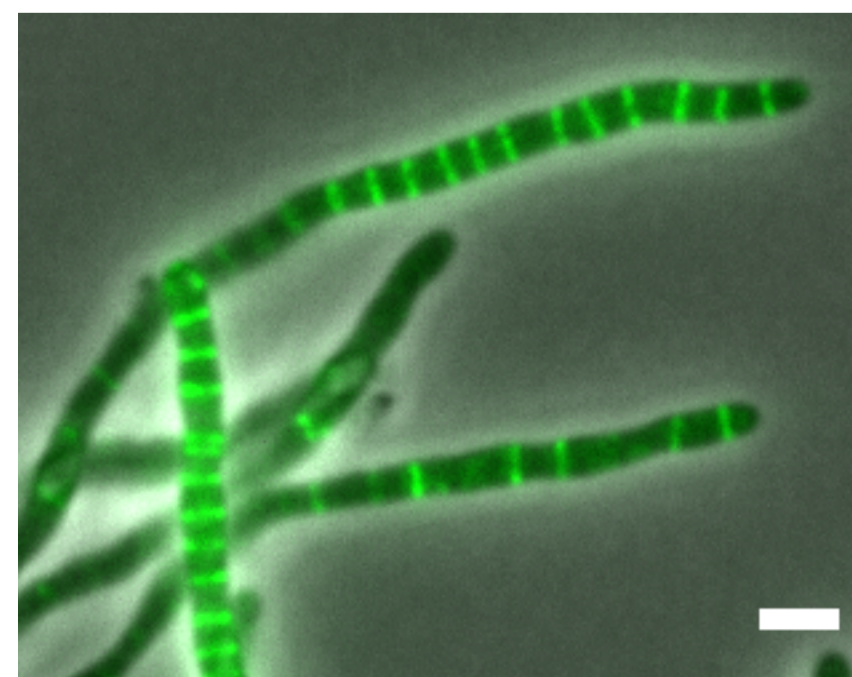
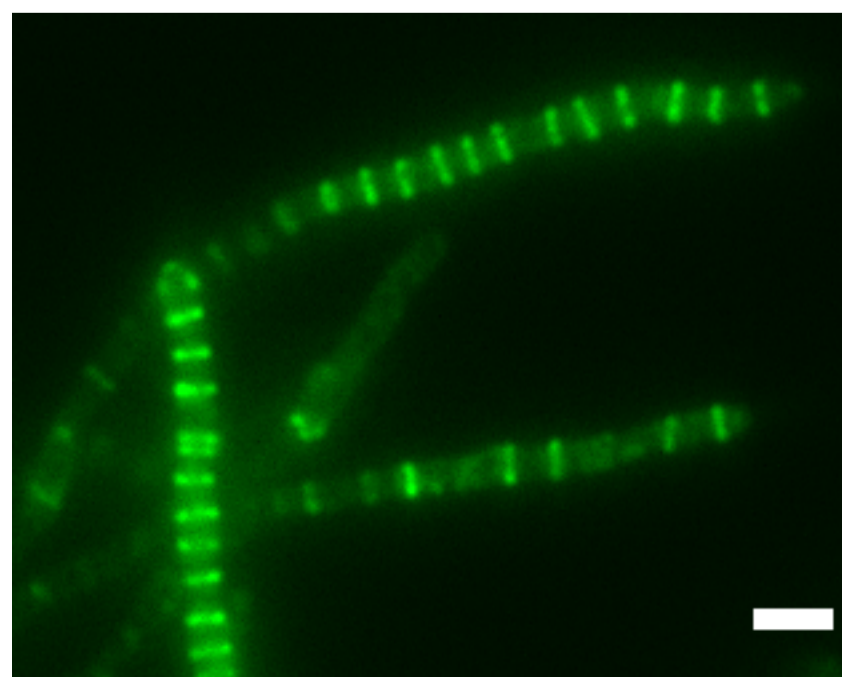
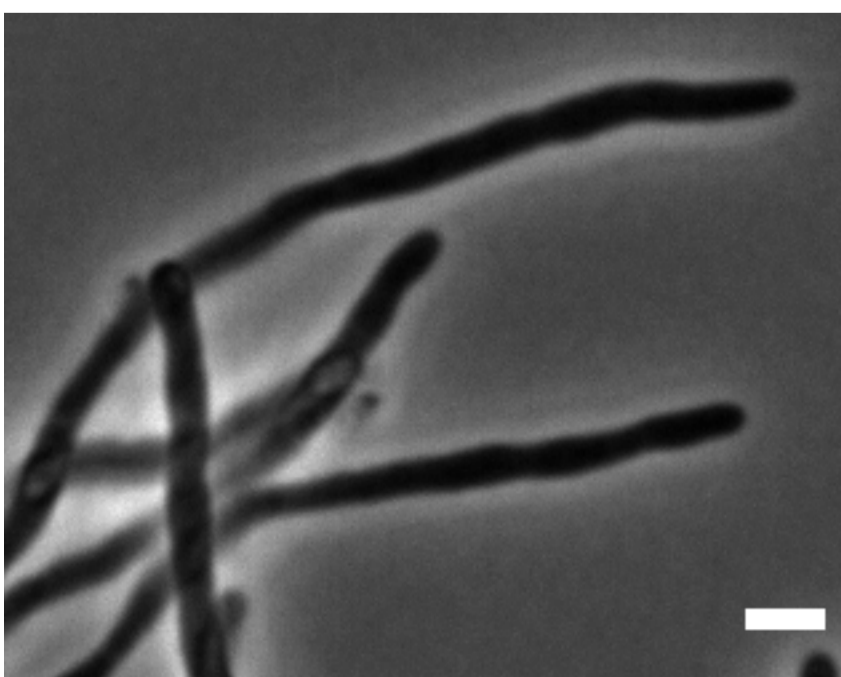




Phase contrast

YPet

Merge

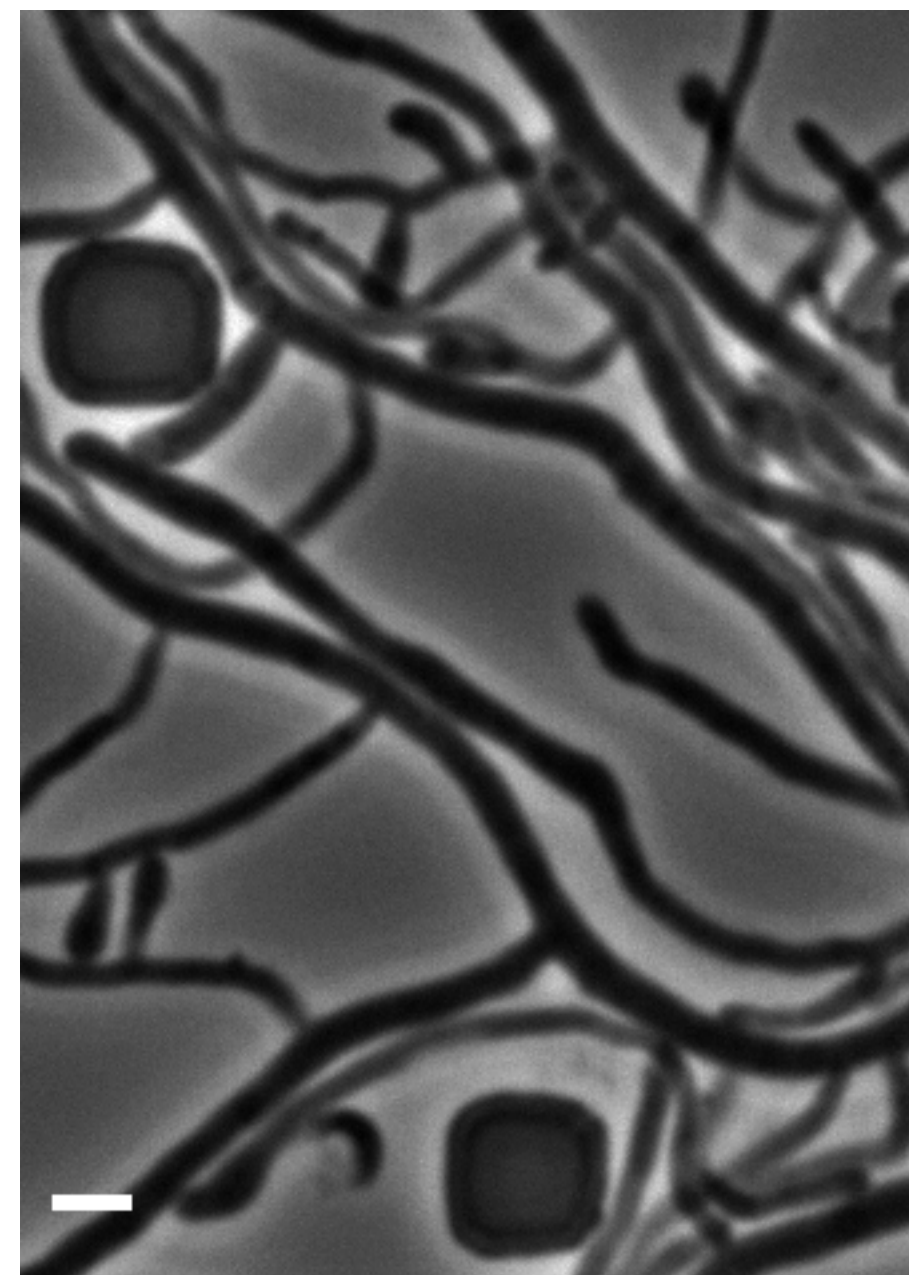
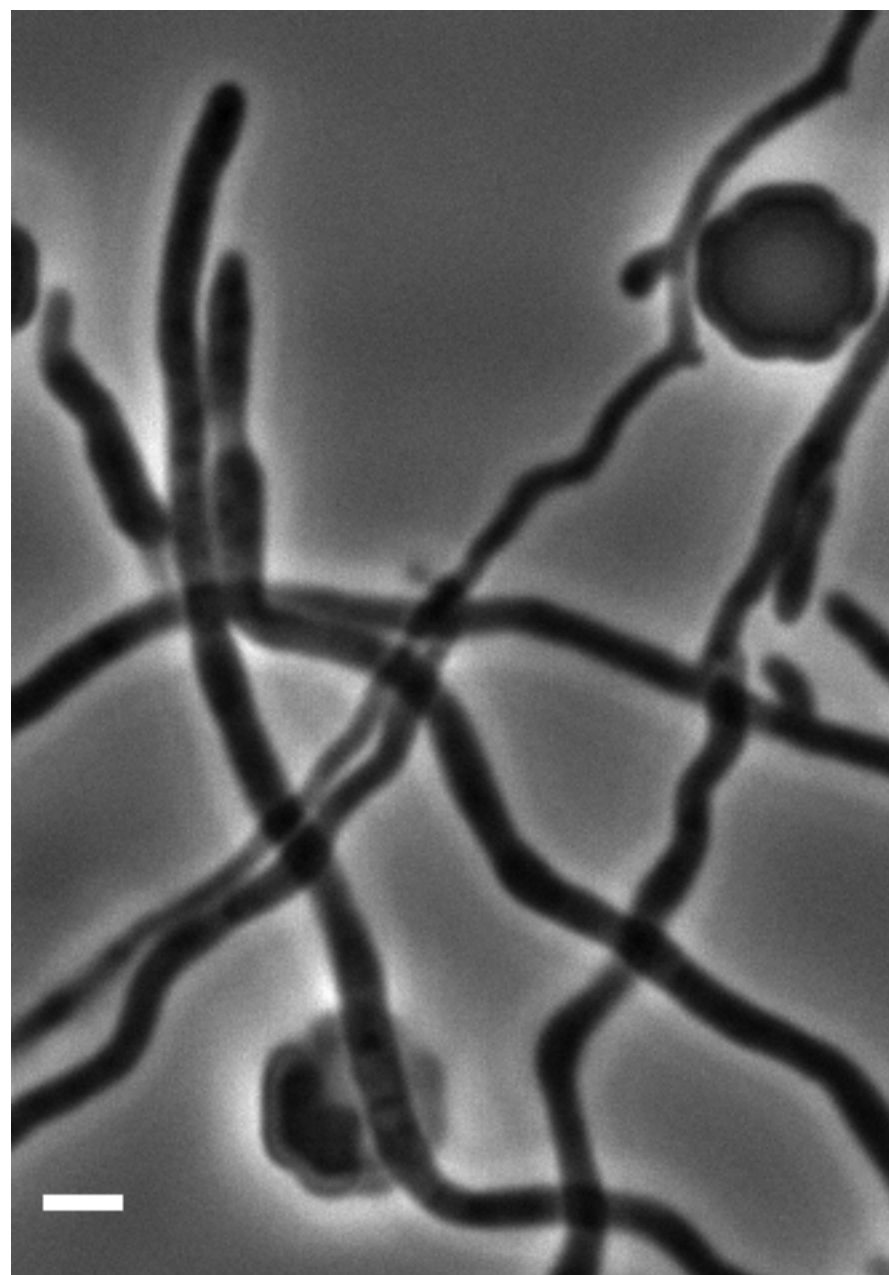
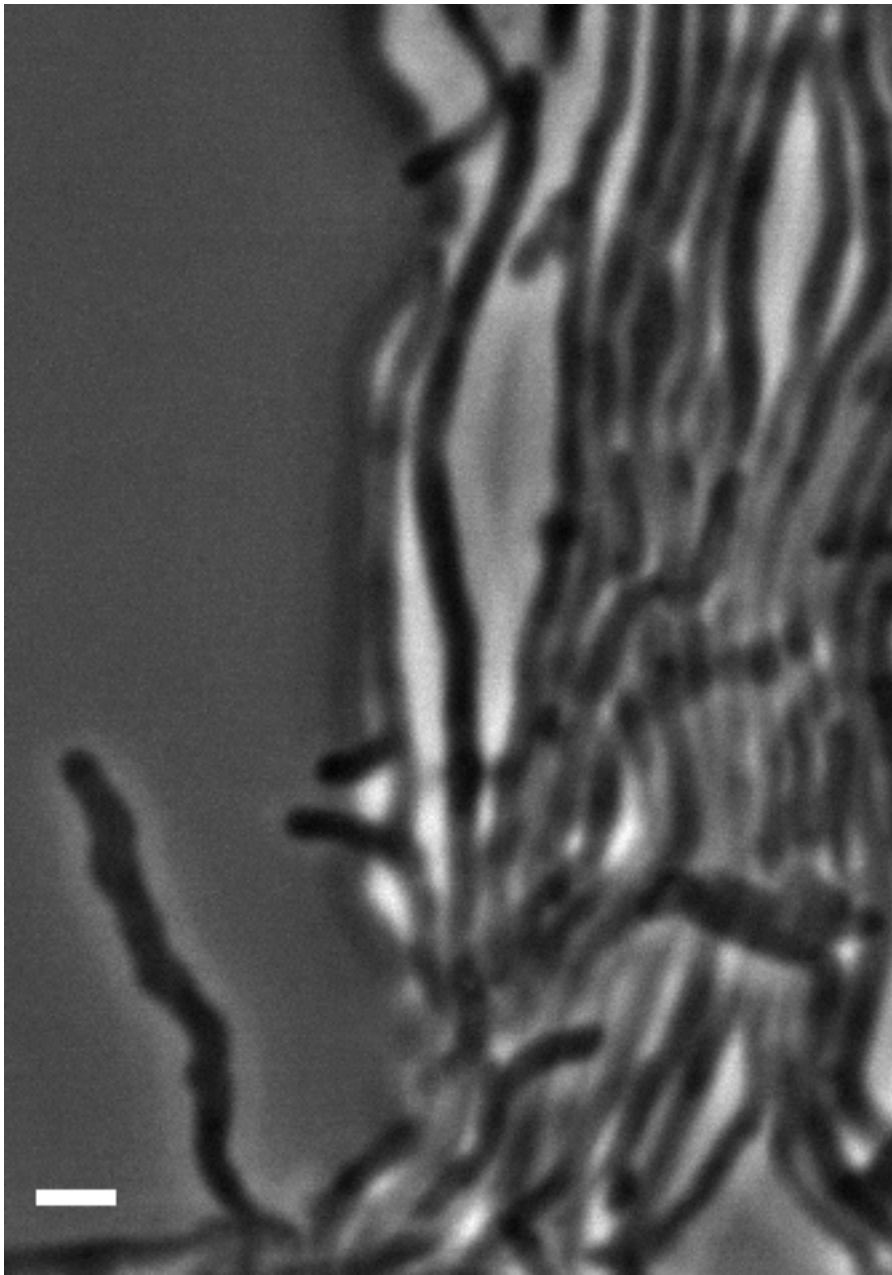
ftsZ⁺/ftsZ-ypet*ΔftsQ/ftsZ-ypet**ΔftsL/ftsZ-ypet**ΔdivIC/ftsZ-ypet**ΔftsW/ftsZ-ypet*

ftsZ⁺/ftsZ-ypet

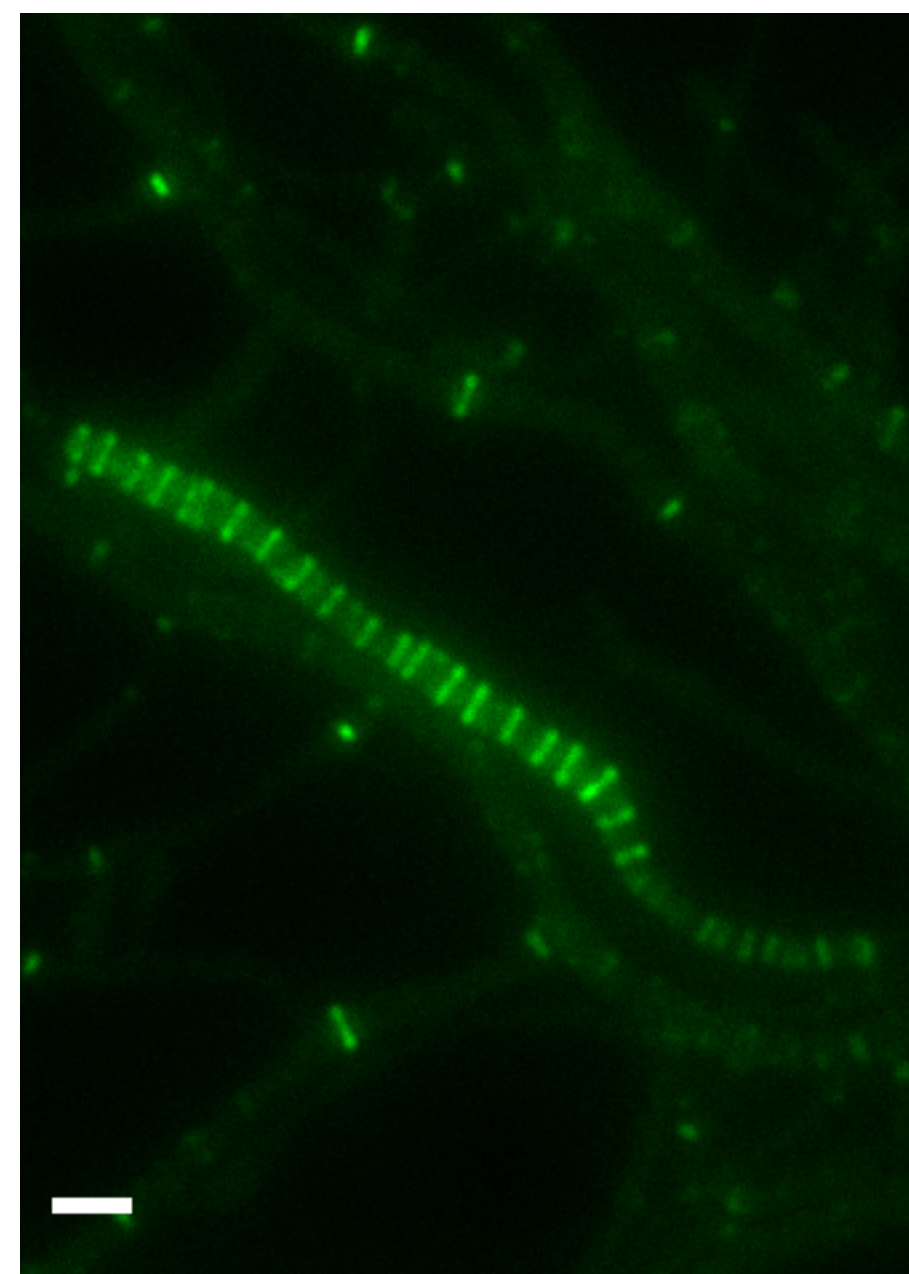
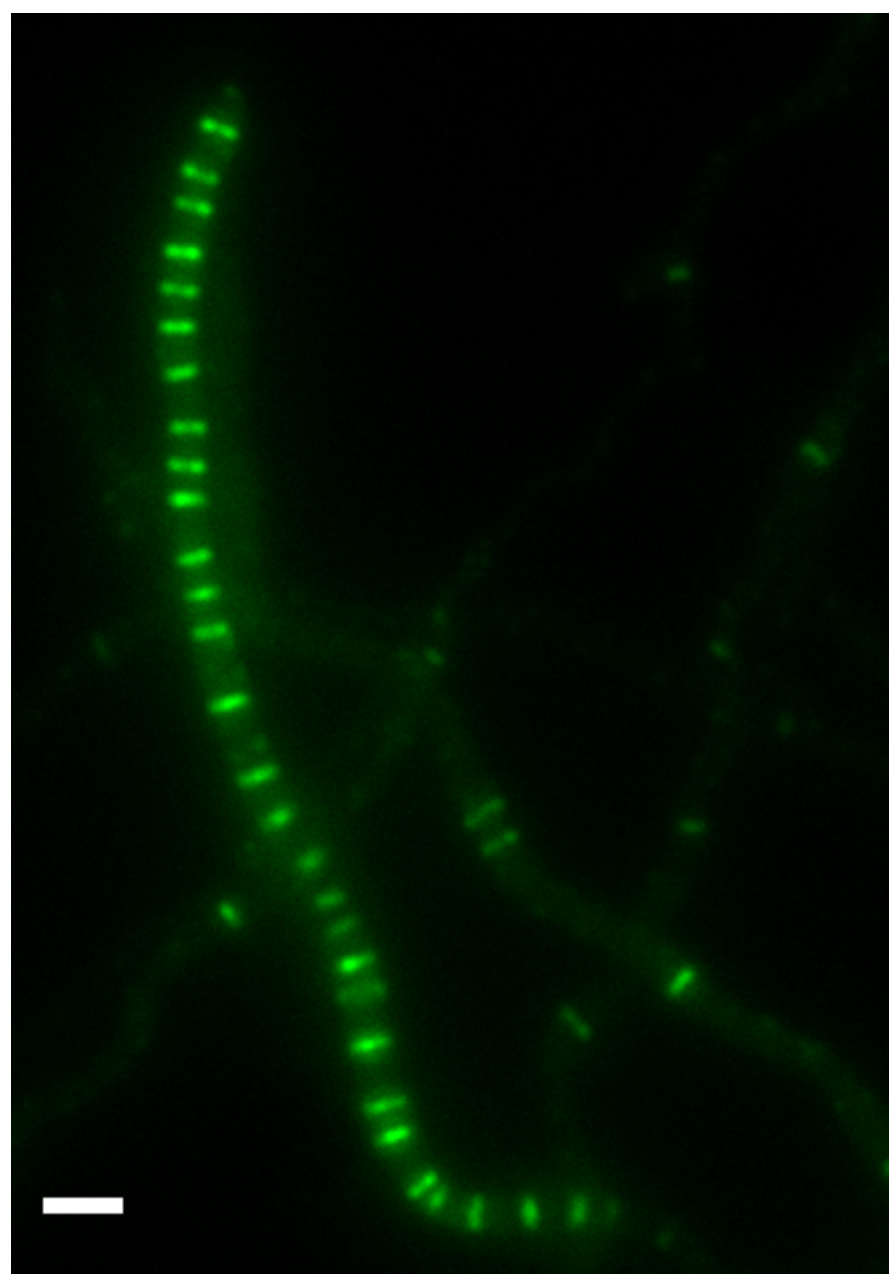
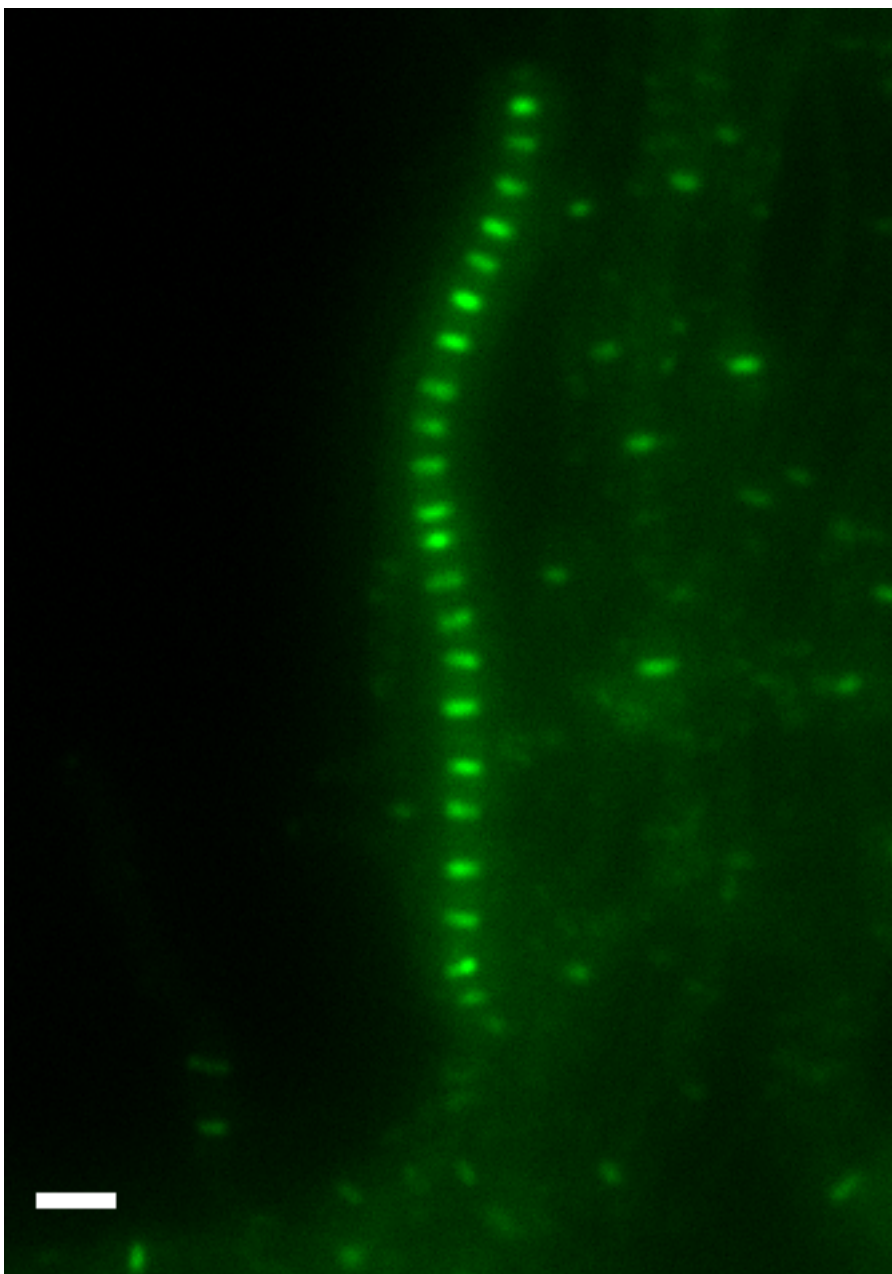
ΔftsQ/ftsZ-ypet

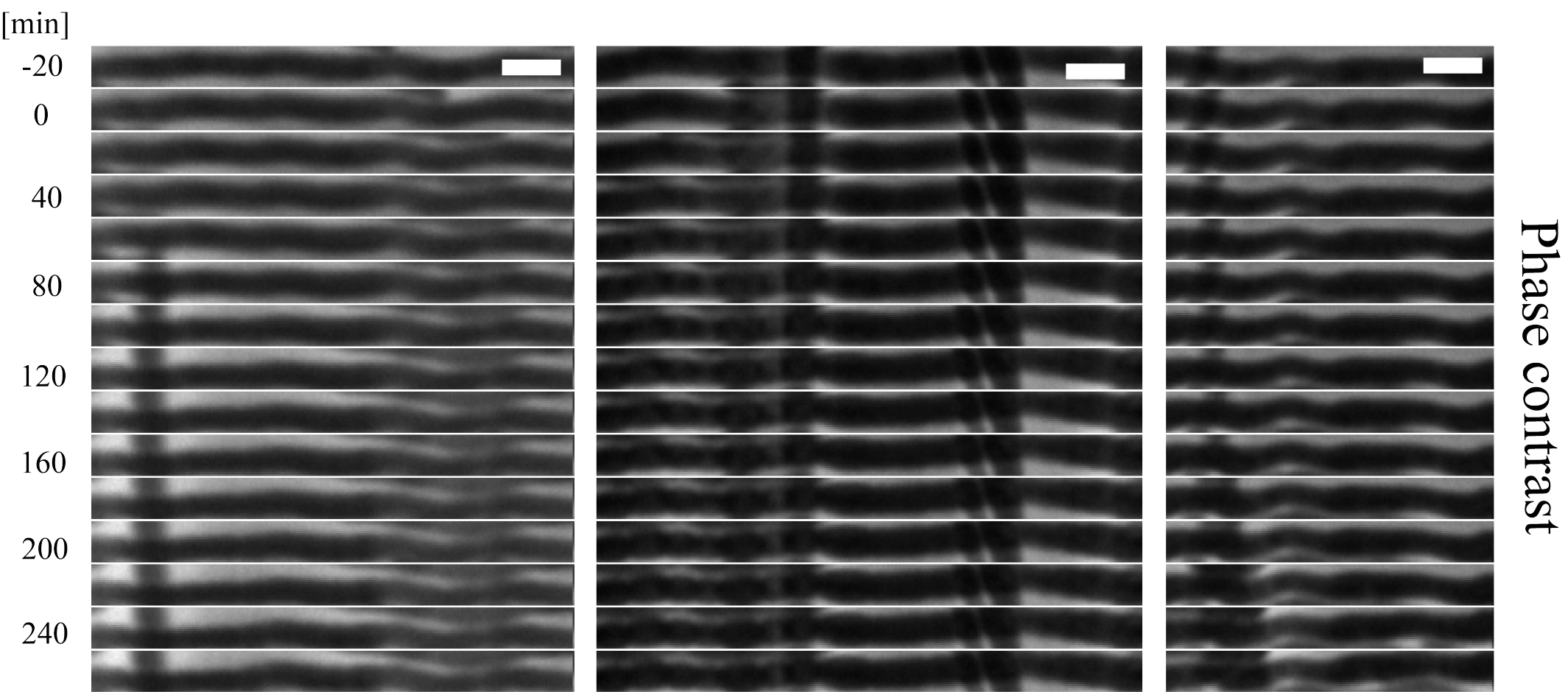
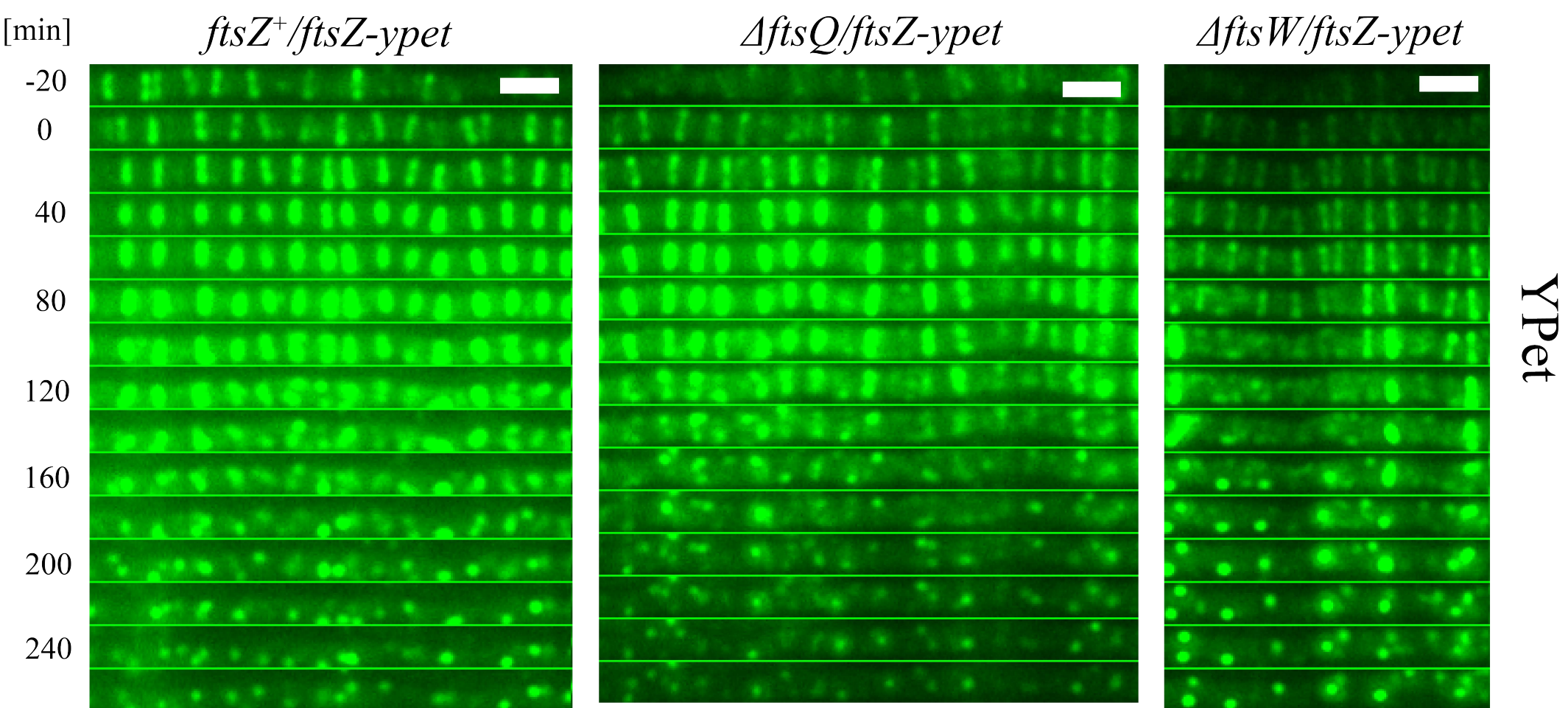
ΔftsW/ftsZ-ypet

Phase contrast



YPet





Supplementary material

Influence of core divisome proteins on cell division in

Streptomyces venezuelae ATCC 10712

Stuart Cantlay^{1,#}, Beer Chakra Sen², Klas Flärdh² and Joseph R. McCormick^{1,*}

¹ *Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA*

² *Department of Biology, Lund University, 223 62 Lund, Sweden*

[#] *Current address: Department of Biological Sciences, West Liberty University, West Liberty, WV 26074, USA*

**Corresponding author*

Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA

Tel: (+1) 412 396 4775

Email: mccormick@duq.edu

Table S1. *S. venezuelae* Strains

Strain Genotypes [†]	ATCC 10712	NRRL B-65442	Comments [‡]
<i>attB</i> _{ΦBT1} ::pMS82	DU522	DU732	cloning vector integrated by site specific recombination, Hyg ^R
<i>attB</i> _{ΦBT1} ::pJS26 (<i>divIC</i> ⁺)	DU620		<i>divIC</i> ⁺ genetic complementation vector integrated <i>in trans</i>
<i>attB</i> _{ΦBT1} ::pJS7 (<i>ftsIL</i> ⁺)	DU528		<i>ftsIL</i> ⁺ genetic complementation vector integrated <i>in trans</i>
<i>attB</i> _{ΦBT1} ::pJS10 (<i>ftsL</i> ⁺)	DU538		<i>ftsL</i> ⁺ genetic complementation vector integrated <i>in trans</i>
<i>attB</i> _{ΦBT1} ::pJS32 (<i>Pdcw-ftsQ</i> ⁺)	DU626		<i>ftsQ</i> ⁺ genetic complementation vector integrated <i>in trans</i>
<i>attB</i> _{ΦBT1} ::pJS6 (<i>ftsW</i> ⁺)	DU524		<i>ftsW</i> ⁺ genetic complementation vector integrated <i>in trans</i>
<i>attB</i> _{ΦBT1} ::pJS8 (<i>ftsZ</i> ⁺)	DU534		<i>ftsZ</i> ⁺ genetic complementation vector integrated <i>in trans</i>
<i>attB</i> _{ΦC31} ::pKF280 (<i>ypet</i>)	DU576	DU733	<i>ypet</i> cloning vector integrated by site specific recombination, Apra ^R
<i>attB</i> _{ΦC31} ::pKF351 (<i>ftsZ-ypet</i>)	DU578	DU724	<i>ftsZ-ypet</i> fusion vector integrated <i>in trans</i> by site specific recombination
Δ <i>divIC::apra</i>	DU518	DU519	471 bp deletion of <i>divIC</i> including the start and stop codons
Δ <i>divIC</i>	DU613		471 bp deletion of <i>divIC</i> including the start and stop codons
Δ <i>divIC attB</i> _{ΦBT1} ::pMS82	DU614		cloning vector integrated by site specific recombination in DU613
Δ <i>divIC attB</i> _{ΦBT1} ::pJS26 (<i>divIC</i> ⁺)	DU615		<i>divIC</i> ⁺ genetic complementation vector integrated <i>in trans</i> in DU613
Δ <i>divIC attB</i> _{ΦC31} ::pKF280	DU634		<i>ypet</i> cloning vector integrated by site specific recombination DU613
Δ <i>divIC attB</i> _{ΦC31} ::pKF351 (<i>ftsZ-ypet</i>)	DU635		<i>ftsZ-ypet</i> fusion vector integrated <i>in trans</i> by site specific recombination in DU613
Δ <i>divIC</i> Δ <i>ftsL</i>	DU592		Mutation in DU613 introduced into DU520
Δ <i>divIC</i> Δ <i>ftsL</i> Δ <i>ftsQ</i>	DU628		Mutation in DU629 introduced into DU592
Δ <i>divIC</i> Δ <i>ftsQ</i>	DU636		Mutation in DU613 introduced into DU629
Δ <i>ftsI::apra</i>	DU508	DU509	1,140 bp deletion beginning 60 bp downstream of the <i>ftsI</i> start codon
Δ <i>ftsI::frit</i>	DU679		1,140 bp deletion beginning 60 bp downstream of the <i>ftsI</i> start codon with insertion of 81 bp <i>frit</i> scar
Δ <i>ftsI::frit attB</i> _{ΦBT1} ::pMS82	DU683		cloning vector integrated by site specific recombination in DU679
Δ <i>ftsI::frit attB</i> _{ΦBT1} ::pJS7 (<i>ftsIL</i> ⁺)	DU691		<i>ftsIL</i> ⁺ genetic complementation vector integrated <i>in trans</i> in DU679
Δ <i>ftsI::frit attB</i> _{ΦC31} ::pKF280	DU681		<i>ypet</i> cloning vector integrated by site specific recombination DU679
Δ <i>ftsI::frit attB</i> _{ΦC31} ::pKF351 (<i>ftsZ-ypet</i>)	DU680		<i>ftsZ-ypet</i> fusion vector integrated <i>in trans</i> by site specific recombination in DU679
Δ <i>ftsIL::apra</i>	DU516	DU517	1,817 bp deletion beginning 5 bp downstream of the <i>ftsL</i> start codon through 984 bp downstream of the <i>ftsI</i> start codon
Δ <i>ftsIL::apra attB</i> _{ΦBT1} ::pMS82	DU574		cloning vector integrated by site specific recombination in DU516
Δ <i>ftsIL::apra attB</i> _{ΦBT1} ::pJS7 (<i>ftsIL</i> ⁺)	DU570		pJS7 integrated at the ΦBT1 attachment site in DU516
Δ <i>ftsI::apra</i> Δ <i>ftsW</i>	DU594		Mutation in DU521 introduced into DU508
Δ <i>ftsL::apra</i>	DU510	DU511	352 bp deletion beginning 5 bp downstream of the <i>ftsL</i> start codon
Δ <i>ftsL</i>	DU520		352 bp deletion beginning 5 bp downstream of the <i>ftsL</i> start codon
Δ <i>ftsL attB</i> _{ΦBT1} ::pMS82	DU544		cloning vector integrated by site specific recombination in DU520
Δ <i>ftsL attB</i> _{ΦBT1} ::pJS10 (<i>ftsL</i> ⁺)	DU546		<i>ftsL</i> ⁺ genetic complementation vector integrated <i>in trans</i> in DU520
Δ <i>ftsL attB</i> _{ΦC31} ::pKF280	DU588		<i>ypet</i> cloning vector integrated by site specific recombination DU520
Δ <i>ftsL attB</i> _{ΦC31} ::pKF351 (<i>ftsZ-ypet</i>)	DU590		<i>ftsZ-ypet</i> fusion vector integrated <i>in trans</i> by site specific recombination in DU520
Δ <i>ftsL</i> Δ <i>ftsQ::apra</i>	DU622		Mutation in DU520 introduced into DU502
Δ <i>ftsQ::apra</i>	DU502	DU503	810 bp deletion beginning at the second codon of <i>ftsQ</i>
Δ <i>ftsQ</i>	DU629		810 bp deletion beginning at the second codon of <i>ftsQ</i>
Δ <i>ftsQ attB</i> _{ΦBT1} ::pMS82	DU630		cloning vector integrated by site specific recombination in DU629

$\Delta ftsQ$ attB _{ΦBT1} ::pJS32 (<i>Pdcw-ftsQ</i> ⁺)	DU631		<i>ftsQ</i> ⁺ genetic complementation vector integrated <i>in trans</i> in DU629
$\Delta ftsQ$ attB _{ΦC31} ::pKF280	DU632		<i>ypet</i> cloning vector integrated by site specific recombination DU629
$\Delta ftsQ$ attB _{ΦC31} ::pKF351 (<i>ftsZ-ypet</i>)	DU633		<i>ftsZ-ypet</i> fusion vector integrated <i>in trans</i> by site specific recombination in DU629
$\Delta ftsW::apra$	DU512	DU513	1,076 bp deletion beginning 140 bp downstream of the <i>ftsW</i> start codon
$\Delta ftsW$	DU521		1,076 bp deletion beginning 140 bp downstream of the <i>ftsW</i> start codon
$\Delta ftsW$ attB _{ΦBT1} ::pMS82	DU548		cloning vector integrated by site specific recombination in DU521
$\Delta ftsW$ attB _{ΦBT1} ::pJS6 (<i>ftsW</i> ⁺)	DU550		pJS6 integrated at the ΦBT1 attachment site in DU521
$\Delta ftsW$ attB _{ΦC31} ::pKF280	DU584		<i>ypet</i> cloning vector integrated by site specific recombination DU521
$\Delta ftsW$ attB _{ΦC31} ::pKF351 (<i>ftsZ-ypet</i>)	DU586		<i>ftsZ-ypet</i> fusion vector integrated <i>in trans</i> by site specific recombination in DU521
$\Delta ftsZ::apra$	DU500	DU669 [1]	844 bp deletion beginning 16 bp upstream of the <i>ftsZ</i> start codon
$\Delta ftsZ::frit$	DU665		844 bp deletion beginning 16 bp upstream of <i>ftsZ</i> with insertion of 81 bp <i>frit</i> scar
$\Delta ftsZ::apra$ attB _{ΦBT1} ::pMS82	DU637	DU671	cloning vector integrated by site specific recombination in DU500/DU699
$\Delta ftsZ::apra$ attB _{ΦBT1} ::pJS8 (<i>ftsZ</i> ⁺)	DU536	DU670	<i>ftsZ</i> ⁺ genetic complementation vector integrated <i>in trans</i> in DU500/DU699
<i>ftsZ2p::apra</i>	DU504	DU505	6 bp deletion of the -10 site (TAGTGT) of a developmentally regulated promoter
<i>ftsZΔ2p</i>	DU523		TAGTGT of -10 site of <i>ftsZ2p</i> replaced with ACTAGA
<i>ftsZΔ2p</i> attB _{ΦBT1} ::pMS82	DU552		cloning vector integrated by site specific recombination in DU523
<i>ftsZΔ2p</i> attB _{ΦBT1} ::pJS8 (<i>ftsZ</i> ⁺)	DU554		<i>ftsZ</i> ⁺ genetic complementation vector integrated <i>in trans</i> in DU523

† Unmarked gene deletions contain an in-frame six base scar (ACTAGA)

‡ All strains were made for this study.

Table S2. Cosmids and plasmids used in the study

Vector/ construct	Description [†]	Reference or source
Sv-4-G01	Source of <i>ftsI</i> , <i>ftsL</i> , <i>ftsW</i> , <i>ftsQ</i> , and <i>ftsZ</i> (division and cell wall locus, <i>dcw</i>)	M. Bibb, unpublished
Sv-5-C06	Source of <i>divIC</i>	M. Bibb, unpublished
pIJ773	Source of <i>oriT apra</i> cassette	[2]
pIJ799	Source of cassette with <i>oriT apra</i> flanked with <i>bla</i> homology	[2]
pKF280	Control plasmid with promoter-less <i>ypet</i> , integrates at <i>attB</i> _{ΦC31} (Apra ^R)	[3]
pKF351	Derivative of pKF280 containing <i>ftsZ-ypet</i>	[3]
pMS82	Cloning vector for genetic complementation, integrates at <i>attB</i> _{ΦBT1} (Hyg ^R)	[4]
pJK1	Δ <i>ftsZ::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK3	<i>ftsZ</i> Δ 2p:: <i>apra-oriT</i> , Sv-4-G01 derivative	This study
pJK5	Δ <i>ftsI::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK6	Δ <i>ftsL::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK7	Δ <i>ftsW::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK9	Δ <i>ftsIL::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK23	<i>ftsZ</i> Δ 2p <i>bla::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK26	Δ <i>ftsL bla::apra-oriT</i> , Sv-4-G01 derivative	This study
pJK27	Δ <i>ftsW bla::apra-oriT</i> , Sv-4-G01 derivative	This study
pJS6	pMS82 containing <i>ftsW</i> (5,434 bp <i>PvuII</i> fragment)	This study
pJS7	pMS82 containing <i>ftsI</i> (5,686 bp <i>PvuII</i> fragment)	This study
pJS8	pMS82 containing <i>ftsZ</i> (3,900 bp <i>BamHI-PstI</i> fragment)	This study
pJS10	pMS82 containing <i>ftsL</i> (2,869 bp <i>KpnI</i> fragment)	This study
pJS18	Δ <i>divIC::apra-oriT</i> , Sv-4-C06 derivative	This study
pJS24	Δ <i>divIC bla::apra-oriT</i> , Sv-4-C06 derivative	This study
pJS26	pMS82 containing <i>divIC</i> (679 bp PCR fragment beginning 187 bp upstream of <i>divIC</i>)	This study
pJS30	Δ <i>ftsQ::apra-oriT</i> , Sv-4-G01 derivative	This study
pJS32	pMS82 carrying <i>P</i> _{<i>dcw</i>} - <i>ftsQ</i> (1095 bp PCR fragment containing 257 bp of <i>P</i> _{<i>dcw</i>} and <i>ftsQ</i>)	This study
pJS39	Δ <i>ftsQ bla::apra-oriT</i> , Sv-4-G01 derivative	This study
pJS40	Δ <i>ftsZ::frit bla::apra-oriT</i> , Sv-4-G01 derivative	This study
pJS41	Δ <i>ftsI::frit bla::apra-oriT</i> , Sv-4-G01 derivative	This study

[†]Apr^R, apramycin resistance; Hyg^R, Hygromycin resistance; Unmarked gene deletions contain an in-frame six base scar (ACTAGA)

Table S3. Oligonucleotides used in this study

Name	Sequence	Application
SVftsZ60	GCGGCGGAACCAACGCGGCGGCGACGACACGTA ACTCGAGATTCCGGGGATCCGTCGACC	<i>ftsZ</i> deletion in cosmid Sv-4-G01
SVftsZ59	TGATGTTGGCCTCGGGGTGGGCGGCCTCGCTGAC CAGCTGTGTAGGCTGGAGCTGCTTC	
SV2pUP66	AGGTTTCGGCGTGTTCGTTGAACGTGCGCCACTTG TCGACTACTAGT ATTCCGGGGATCCGTCGACC	<i>ftsZ2p</i> “-10” deletion in cosmid Sv-4-G01
SV2pDOWN65	GGTACCAGTGTCTCTGTTTCGCTGGACTCTTCCG AACAGG TCTAGA TGTAGGCTGGAGCTGCTTC	
SVftsL64	CGGGGCGCCGAGCGGATCCGGGAAGACGTCCAG TGAGCA CTAGT ATTCCGGGGATCCGTCGACC	<i>ftsL</i> deletion in cosmid Sv-4-G01
SVftsL65	CCGGCTTCGAGGGCGTCGGGGACGGCTTCGGGG CCTCCG CTCTAGAT TGTAGGCTGGAGCTGCTTC	
SVftsW64	TCCACGAGCGGGCGCGGAAGGCCTGGGACCGGC CGCTCA CTAGT ATTCCGGGGATCCGTCGACC	<i>ftsW</i> deletion in cosmid Sv-4-G01
SVftsW63	CGGCGCAGGGCCAGGGCCGCTTTCGCGGCGGGT TCCTG TCTAGAT TGTAGGCTGGAGCTGCTTC	
SVftsI60	GCGCCGCCGCGTTCGCCGACCCGCCCGGCCCGC GCGCCCCATTCCGGGGATCCGTCGACC	<i>ftsI</i> deletion in cosmid Sv-4-G01
SVftsI59	GCGTCAGGTACCAGGTCTCGTGGTCGACGTCGTC CTTGAATGTAGGCTGGAGCTGCTTC	
ftsQSXFor	AGGCGTAGCGCGGGCGGCTGAGGGCAGGAGGCGC CAGGTG ACTAGT ATTCCGGGGATCCGTCGACC	<i>ftsQ</i> deletion in cosmid Sv-4-G01
ftsQSXRev	CTGGCCAACCAGGGTGCTGGCCAGGGGTGATAC CCGTCAT CTAGAT TGTAGGCTGGAGCTGCTTC	
divICSXF	GTGCGGGGACGTCCGCGTGAACAGGGGAGGCGA CACGACA CTAGT ATTCCGGGGATCCGTCGACC	<i>divIC</i> deletion in cosmid Sv-5-C06
divICSXR	TTCTCTCGGTTGCCTTGCTCGTCTGGTGC GGAGG AGGG TCTAGAT TGTAGGCTGGAGCTGCTTC	
SVdcwPSXF	CGCTCGCCCGTACCTCCGCGCCGGATCTGAGAG GGCGCA ACTAGT ATTCCGGGGATCCGTCGACC	Unmarked deletion of <i>dcw</i> locus between <i>Pdcw</i> and <i>ftsQ</i> in cosmid Sv-4-G01
SVdcwPSXQR	CGCGCCGCTCTTCTCGGCGGTCGTCGGTCCGGCT GCC ACTCTAGAT TGTAGGCTGGAGCTGCTTC	
22ftsQEcoRV	GGAATTC GATATCG AGGGGACAAAGAACCGCAT	PCR amplify <i>Pdcw-ftsQ</i> for complementation
22ftsQSpeI	GGAATTC ACTAGT GCTGGCCAGGGGTGATACCC	
divICcompEcoRV	GGAATTC GATATCCAT CGAGGAGATCCTCGAC	PCR amplify <i>divIC</i> with native promoter for complementation
divICcompSpeI	GGAATTC ACTAGT GTTTCTCTCGGTTGCCTTGC	

Figure S1

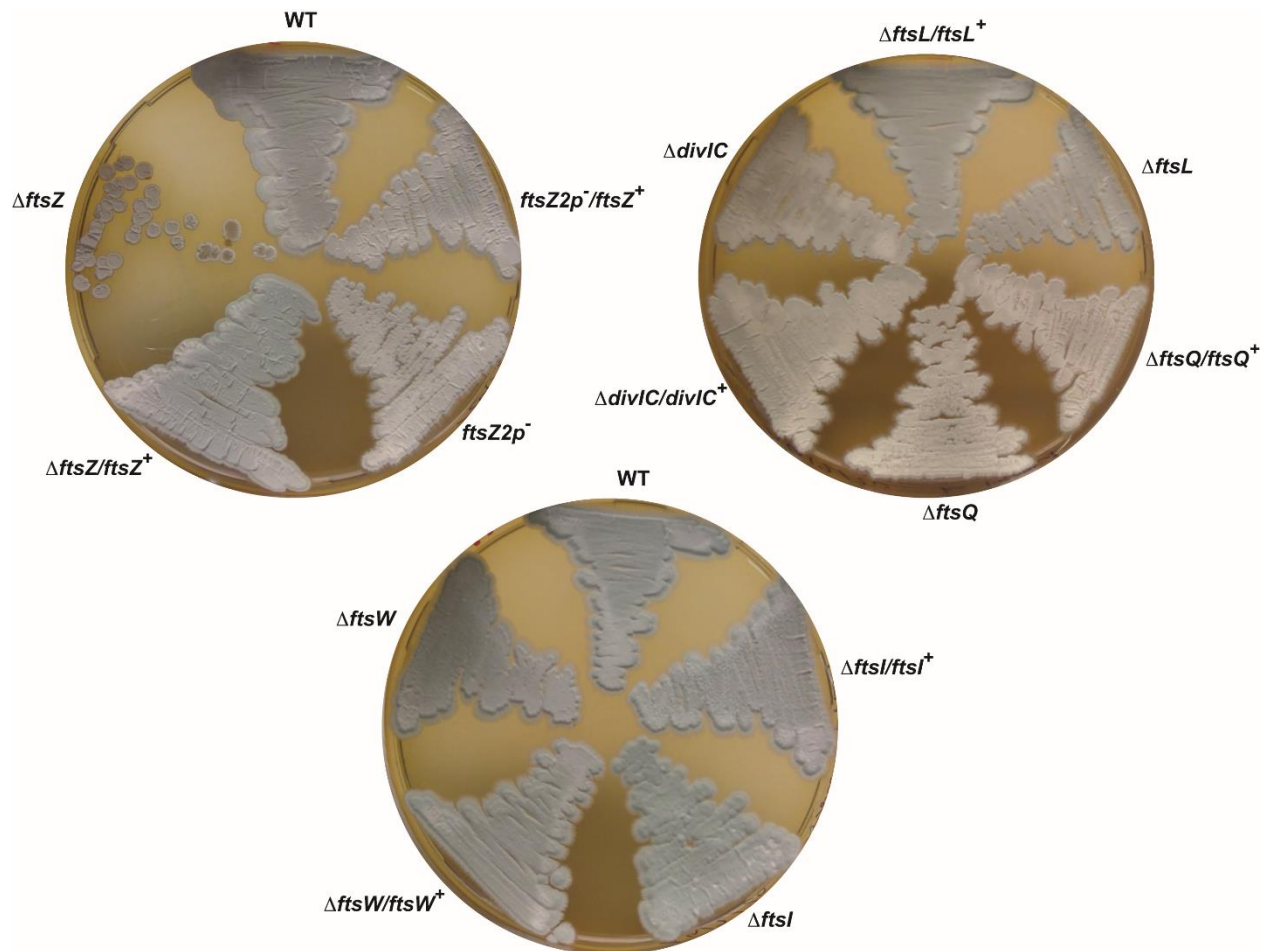


Figure S1. Macroscopic phenotypes of isolated *S. venezuelae* cell division mutants. Shown are patches of wild type *S. venezuelae* ATCC 10712 and congenic divisome mutant strains after growth on MYM agar for 4 days at 30°C. Pairs of mutant strains are shown containing either empty integration vector (pMS82) or a cognate genetic complementation vector, respectively. The wild type parent strain shown also contains the empty vector pMS82 (WT).

Figure S2

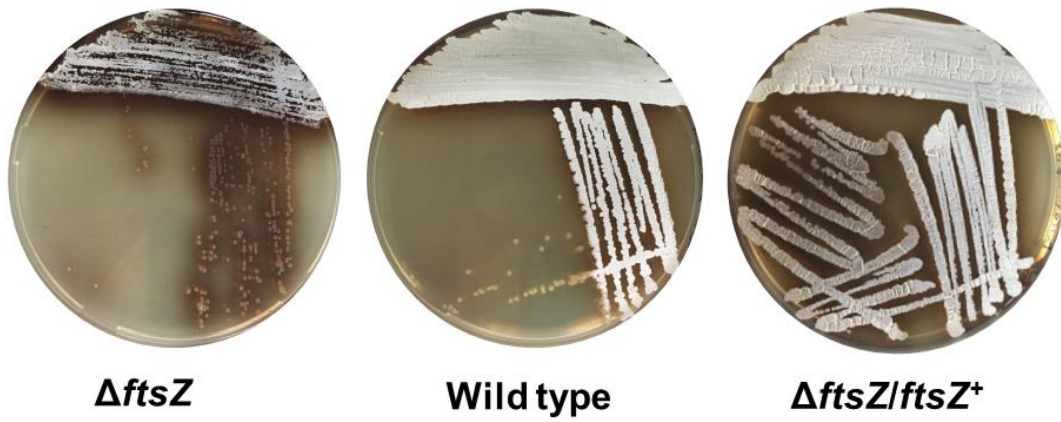


Figure S2. Colony phenotypes of *ftsZ*-null mutant, complemented null mutant and wild type strains.
Shown are streak plates of *ftsZ*-null mutant (DU500), wild type and *ftsZ*-null mutant containing the genetic complementation vector pJS8 (DU537) grown for 2 days on MYM agar at 30°C.

Figure S3

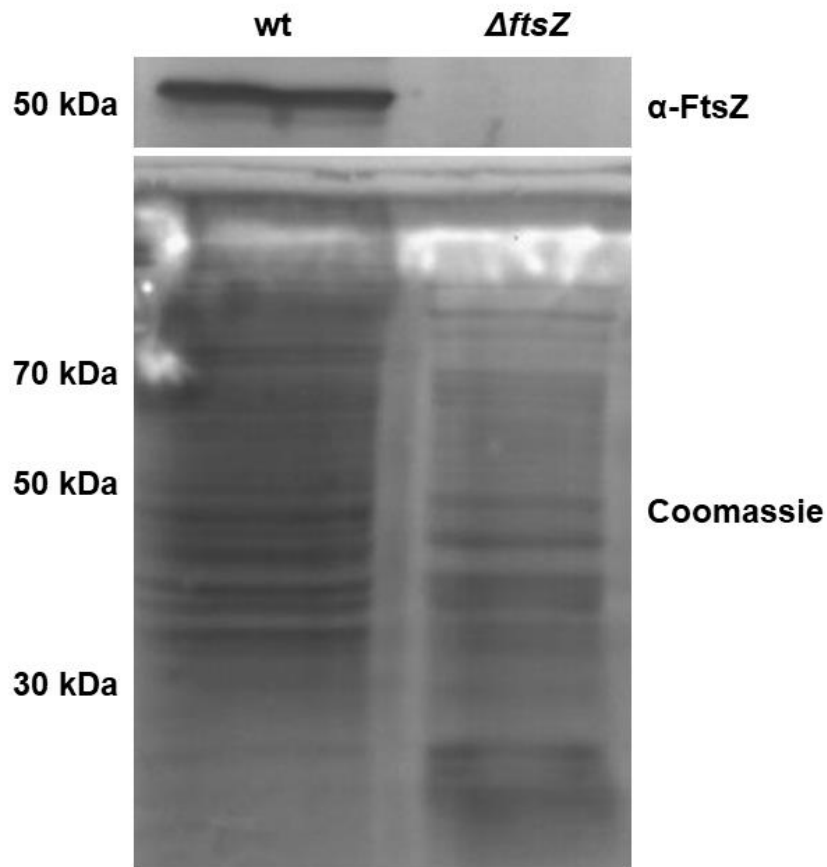


Figure S3. Western blot analysis of wild type and *ftsZ*-null mutant strains using polyclonal anti-FtsZ antibody.

Cultures of wild type and *ftsZ*-null mutant (DU500) were grown overnight in MYM liquid medium. Cell extracts were obtained by bead mill homogenization and normalized amounts of total protein was fractionated in duplicate on 12% polyacrylamide-SDS gel. Fractionated proteins were either stained with Coomassie brilliant blue (lower panel) or transferred to a solid support for a western blot analysis (upper panel). FtsZ was detected using a polyclonal antibody raised against FtsZ from *S. coelicolor* [5] and a secondary antibody conjugated with alkaline phosphatase.

Figure S4

(a)

<i>S. venezuelae</i>	CGGGTATcaccCtGGCCAGCACCTGGTTGGcCAGCGCTACGGgTGATCACATAGGG	58	
<i>S. coelicolor</i>	gaCGGGTATacgtgCaGGCCAGCACCTGGTTGGgCAGCGCTACGGcTGATCACATAGGG	60	
	<i>ftsZ3p</i>		
	##		
<i>S. venezuelae</i>	TGAAAAGAAA AA CGGGAGGTTTCGGCGTGTTCGTTGAACGTGCGCCACTTGTTCGACTTAGT	118	
<i>S. coelicolor</i>	TGAAAAGAAA AA CGGGAGGTTTCGGCGTGTTCGTTGAACGTGCGCCACTTGTTCGACTTAGT	120	
	<i>ftsZ2p</i>	<i>ftsZ1p</i>	
	###	##	
<i>S. venezuelae</i>	GTCCTGT TCG GAAGAGTCCAgcGAACAGAGACACTGGTAACCCTAAACTTCAaCGT TAGG	178	
<i>S. coelicolor</i>	GTCCTGT TCG GAAGAGTCCAagGAACAGACACACTGGTAACCCTAAACTTCAgCGT TAGG	180	
<i>S. venezuelae</i>	GTTtGGGTTCGGCGtTtCGGACCGTCCCAATCGGCATCcGTCGgaGcGgCGCGa	AcC 234	
<i>S. coelicolor</i>	GTTcGGGTTCGGCGcTaCGGACCGTCCCAATCGGCATCaGTCGtcGgGtCGCGggggcAtC	240	
<i>S. venezuelae</i>	AacGCgcgGcgac	GACACGTAACTCGAGGCGAGAGGCCTTCGAC	278
<i>S. coelicolor</i>	AgtGCttcGcgggCCGGGCGACACGTAACTCGAGGCGAGAGGCCTTCGAC	290	

(b)

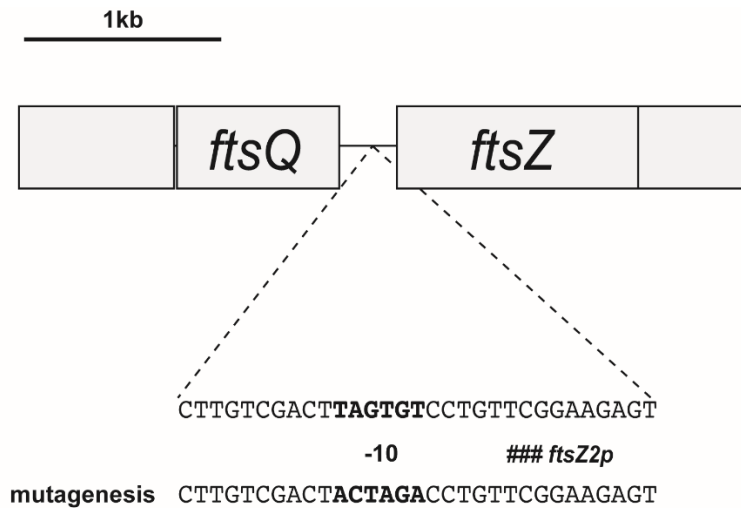


Figure S4. Comparison of the nucleotide sequences for *S. coelicolor* and *S. venezuelae* *ftsQ-ftsZ* intergenic regions containing the developmental *ftsZ2p* promoter and the sequence of the constructed *S. venezuelae* *ftsZ2p* promoter mutation.

(a) Nucleotide sequences of the entire intergenic regions upstream of *ftsZ* from *S. venezuelae* (278 bases) and *S. coelicolor* (290 bases) were aligned and the 3 promoters mapped in *S. coelicolor* are indicated (hash marks and bolded transcription start sites). Conserved sequences are in capital letters and divergent sequences are in small letters. (b) Five of the six residues at the -10 sequence of *ftsZ2p* (TAGTGT) for *S. venezuelae* were mutated to ACTAGA in this study (-10 sequences are bolded). The introduction of this *ftsZΔ2p* mutation into *S. venezuelae* ATCC 10712 created strain DU523.

Figure S5

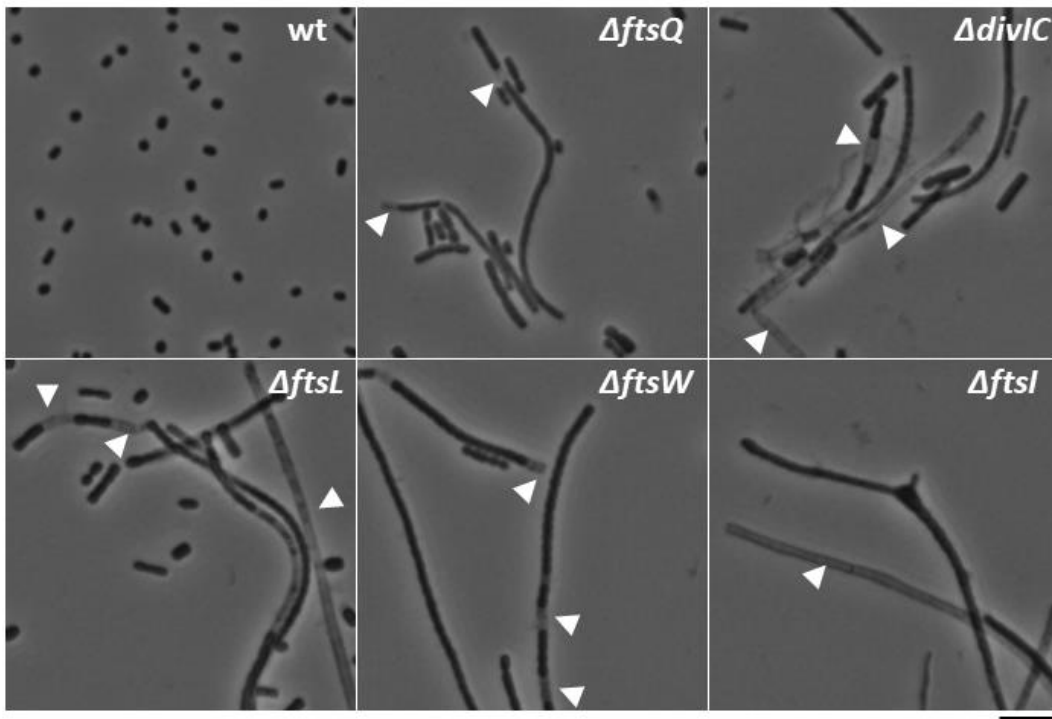


Figure S5. Spores and aerial hyphae are less robust in mutants for *ftsQ*, *ftsL*, *divIC*, *ftsW* and *ftsI* than in the wild type.

Strains were grown for 4 days on MYM agar at 30°C. Impression coverslip lifts were prepared and representative phase-contrast micrographs are shown for wild type and mutant strains. Arrowheads indicate frequent lysed regions of hyphae and spore compartments for the mutants, which are not typically observed for the wild type. Scale bar, 5 μ m.

Figure S6

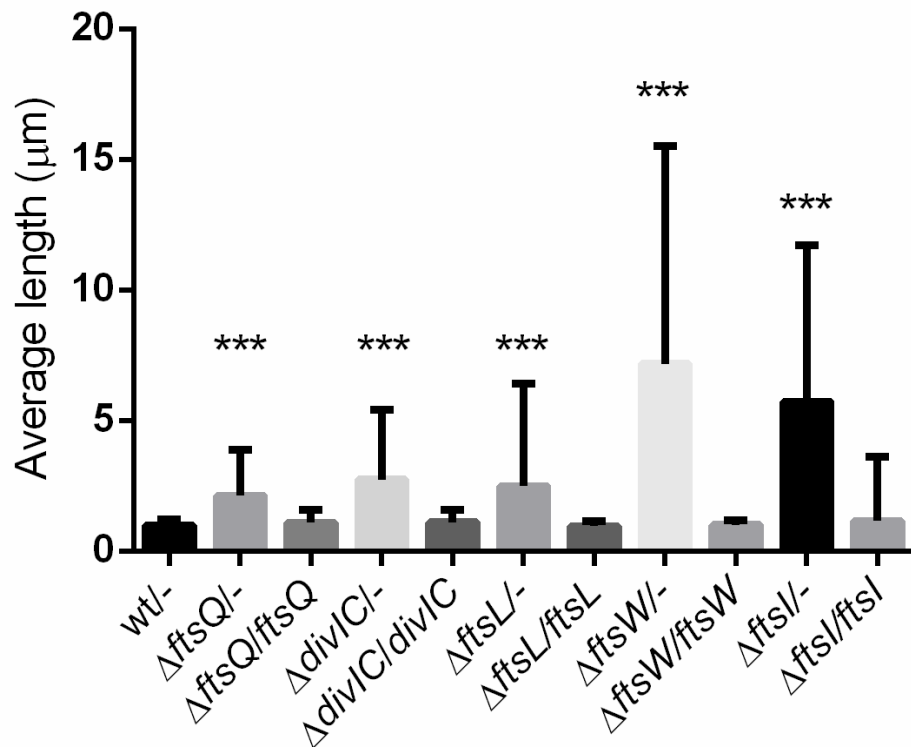


Figure S6. The average length of mature spore and spore-like cells resulting from completed development-associated division events from wild type and mutant strains. Strains were grown for 4 days on MYM agar and spores and spore-like cells were harvested in a standard fashion. Samples for the wild type, containing pMS82 (empty vector), and mutant strains, containing either pMS82 or a genetic complementation vector containing the cognate divisome gene, were spotted onto pads of 1% agarose and images were captured by phase-contrast microscopy. The lengths of spores and spore-like cells were measured. The data represent averages from 3 technical replicates. Bars show the standard deviation. For each strain, N = 750. A one-way ordinary ANOVA analysis with Dunnett's multiple comparisons test showed that the mutants all differ from the wild type with $p < 0.0001$. The average spore length of each divisome mutant is significantly different from the wild type while the average spore length of each complemented strain is not.

Figure S7

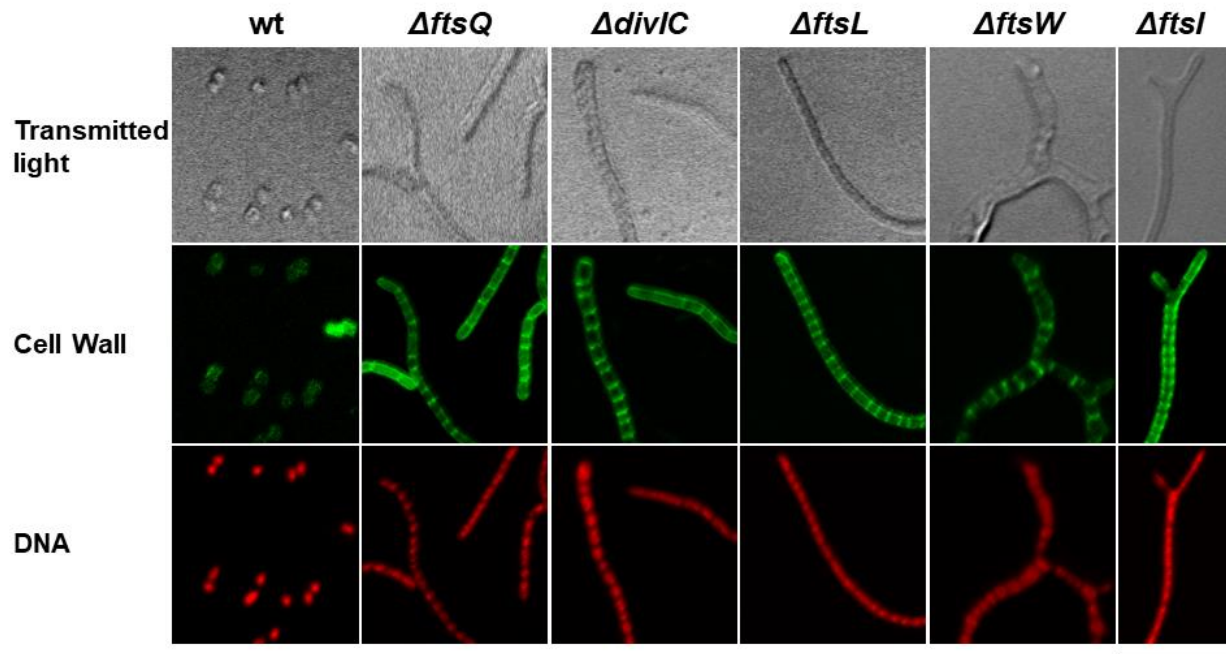


Figure S7. Developmental genome segregation is not overtly affected in core divisome mutants.

Strains were grown for 2 days on MYM agar at 30°C and impression coverslip lifts were made. The top row contains corresponding DIC light images. Samples of aerial hyphae were stained for cell wall (green) and DNA (red) and viewed by epifluorescence microscopy. Wild type samples contained mainly spores. Examples of aerial hyphae of mutant strains are shown. Aerial hyphae of the mutants typically contain segregated nucleoids with few anucleate compartments. Scale bar represents 5 μ m.

Figure S8

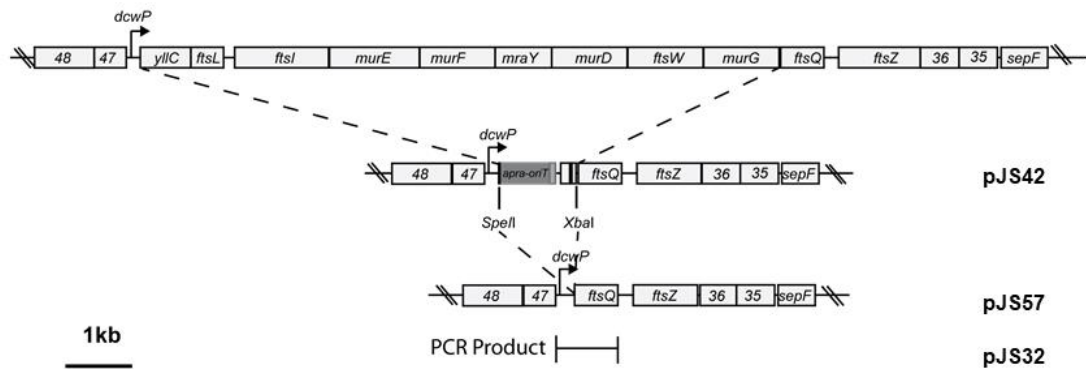


Figure S8. Intermediate plasmid constructions used to make a complementation plasmid for a $\Delta ftsQ$ mutant.

A complementation plasmid was constructed to put expression of the *ftsQ* gene directly under the control of the native *dcw* locus promoter (pJS32). Starting with cosmid Sv-4-G01, recombineering was used to delete 9 *dcw* genes located between *ftsQ* and the *dcw* promoter generating pJS42. The unique *SpeI* and *XbaI* restriction sites, introduced in the process of making pJS42, were digested and religated resulting in pJS57. pJS57 was used as template for PCR to generate complementation plasmid pJS32.

Movie legends

Movie S1: Time-lapse fluorescence microscopy of FtsZ-YPet in sporulating wild-type *S. venezuelae*. Shown is a time-lapse microscopy experiment consisting of fluorescence images of FtsZ-YPet (right) and the corresponding phase-contrast images (left) of DU578 (*ftsZ*⁺ *attB*_{ϕC31}::pKF351[*P*_{*ftsZ*}-*ftsZ-ypet*]). Cells were grown at 30°C in MYM medium in a microfluidic system and monitored by fluorescence microscopy. After an initial period (4-6 hours) of vegetative growth, spent medium was administered to induce sporulation. The time interval between each frame is 10 min. Experiment was run two times. Scale bar, 2 μm.

Movie S2: Time-lapse fluorescence microscopy of FtsZ-YPet in sporulating *S. venezuelae* *ΔftsQ* mutant. Shown is a time-lapse microscopy experiment consisting of fluorescence images of FtsZ-YPet (left) and the corresponding phase-contrast images (right) of DU633 (*ΔftsQ* *attB*_{ϕC3}::pKF351[*P*_{*ftsZ*}-*ftsZ-ypet*]). Cells were grown at 30°C in MYM medium in a microfluidic system and monitored by fluorescence microscopy. After an initial period (4-6 hours) of vegetative growth, spent medium was administered to induce sporulation. The time interval between each frame is 10 min. Experiment was run two times. Scale bar, 5 μm.

Movie S3: Time-lapse fluorescence microscopy of FtsZ-YPet in sporulating *S. venezuelae* *ΔftsW* mutant. Shown is a time-lapse microscopy experiment consisting of fluorescence images of FtsZ-YPet (right) and the corresponding phase-contrast images (left) of DU586 (*ΔftsW* *attB*_{ϕC31}::pKF351[*P*_{*ftsZ*}-*ftsZ-ypet*]). Cells were grown at 30°C in MYM medium in a microfluidic system and monitored by fluorescence microscopy. After an initial period (4-6 hours) of vegetative growth, spent medium was administered to induce sporulation. The time interval between each frame is 10 min. Experiment was run two times. Scale bar, 5 μm.

Supplementary references

1. **Santos-Beneit F, Roberts DM, Cantlay S, McCormick JR, Errington J.** A mechanism for FtsZ-independent proliferation in *Streptomyces*. *Nat Commun.* 2017;8:1378. Doi: 10.1038/s41467-017-01596-z
2. **Gust B, Challis GL, Fowler K, Kieser T, Chater KF.** PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci USA.* 2003;100:1541-6. Doi: 10.1073/pnas.0337542100
3. **Donczew M, Mackiewicz P, Wrobel A, Flårdh K, Zakrzewska-Czerwinska J, Jakimowicz D.** ParA and ParB coordinate chromosome segregation with cell elongation and division during *Streptomyces* sporulation. *Open Biol.* 2016;6:150263. Doi: 10.1098/rsob.150263
4. **Gregory MA, Till R, Smith MC.** Integration site for *Streptomyces* phage phiBT1 and development of site-specific integrating vectors. *J Bacteriol.* 2003;185:5320-3. Doi: 10.1128/jb.185.17.5320-5323.2003
5. **Schwedock J, McCormick JR, Angert EA, Nodwell JR, Losick R.** Assembly of the cell division protein FtsZ into ladder-like structures in the aerial hyphae of *Streptomyces coelicolor*. *Mol Microbiol.* 1997;25:847-58. Doi: 10.1111/j.1365-2958.1997.mmi507.x