JOURNAL OF BACTERIOLOGY, Jan. 2009, p. 661–664 Vol. 191, No. 2
0021-9193/09/$08.00+0 doi:10.1128/JB.01048-08

NOTES

Medium-Dependent Phenotypes of *Streptomyces coelicolor* with Mutations in *ftsI* or *ftsW*▽†

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Received 28 July 2008/Accepted 24 October 2008

***Streptomyces coelicolor* A3(2) *ftsI*- and *ftsW*-null mutants produced aerial hyphae with no evidence of septation when grown on a traditional osmotically enhanced medium. This phenotype was partially suppressed when cultures were grown on media prepared without sucrose. We infer that functional FtsZ rings can form in *ftsI*- and *ftsW*-null mutants under certain growth conditions.**

Rod-shaped bacteria that produce a peptidoglycan cell wall synthesize lateral-wall material during cell elongation and produce septa during cytokinesis. Most rod-shaped bacteria possess separate systems for these processes, each containing a protein of the SEDS (*s*hape, *e*longation, *d*ivision, and *s*porulation) family and a cognate class B penicillin-binding protein (PBP) (7, 9, 11). In *Escherichia coli*, the protein pairs involved in cell elongation and division are RodA-PBP2 and FtsW-FtsI (PBP3), respectively. However, some bacteria possess three protein pairs, as in *Bacillus subtilis*, where sporulation-specific division genes exist in addition to those for vegetative functions (15). *Streptomyces coelicolor* is a gram-positive, filamentous bacterium that requires cell division only for sporulation (13). Its genome possesses four homologous SEDS-PBP pairs (3).

Here we report the characterization of *S. coelicolor* cell division genes *ftsI* and *ftsW*. We show that *ftsI* and *ftsW* are dispensable for colony formation but are required for efficient cell division. Similar to the *ftsL* and *divIC* mutants (2), the *ftsI*- and *ftsW*-null mutants displayed medium-dependent phenotypic defects that are more severe on an osmotically enhanced medium. We suggest that because the *ftsI* and *ftsW* mutants are able to divide when grown on certain media, other proteins may compensate for the loss of FtsI and FtsW. Chains of spores are produced under certain growth conditions, implying that ladder-like arrays of Z rings (18) must be stably formed and function in the absence of FtsI and FtsW under certain conditions.

Identification of *ftsI* and *ftsW* homologues in *S. coelicolor*. The *S. coelicolor ftsI* and *ftsW* homologues, $ftsI_{Sc}$ (StrepDB [http://streptomyces.org.uk/] accession number SCO2090) and $ftsW_{Sc}$ (accession number SCO2085), are located in the division and cell wall (*dcw*) cluster (Fig. 1). We determined the gene sequences prior to the *S. coelicolor* genome project. $ftsI_{Sc}$ is predicted to encode a 654-amino-acid, 69.5-kDa bitopic membrane protein with 26% (160/602) of its residues identical to *B. subtilis* PBP 2B (the FtsI homologue), 31% (188/604) identical to *B. subtilis* SpoVD (the sporulation-specific FtsI homologue), and 29% (175/586) identical to *E. coli* FtsI. $ftsW_{Sc}$ is predicted to encode a 456-amino-acid, 48-kDa integral membrane protein with 36% (128/351) of its residues identical to *B. subtilis* SpoVE (the sporulation-specific FtsW homologue), 36% (137/373) identical to *B. subtilis* FtsW (YlaO), and 31% (114/358) identical to *E. coli* FtsW. $FtsW_{Sc}$ lacks the unique C-terminal extension required for interaction with FtsZ in the related actinomycete *Mycobacterium tuberculosis* (5), and the three other *S. coelicolor* SEDS homologues have even shorter C termini. Thus, assignments for $ftsW_{Sc}$ and $ftsI_{Sc}$ are based on sequence similarity and synteny in the *dcw* cluster, as well as on mutant phenotypes like those of *S. coelicolor ftsL* and *divIC* mutants (2). A similar analysis of the same genes was concurrently reported (14).

***ftsI*$_{Sc}$ and *ftsW*$_{Sc}$ are dispensable, and mutants have medium-dependent phenotypes.** We constructed a C69-derived cosmid (16) called pBP10 by using in vivo recombination in *E. coli* containing a deletion-insertion mutation (8), in which an internal 1.3-kb fragment of $ftsW_{Sc}$ (codons 5 to 450 of 456) was replaced by an apramycin resistance cassette (Fig. 1). This cosmid was introduced by conjugation into wild-type *S. coelicolor* strain M145 (Table 1), independently isolated apramycin-resistant, kanamycin-sensitive marker replacement strains were obtained, and one representative was named PFB22 [Δ*ftsW*::*acc*(*3*)IV]. Therefore, like *ftsZ*, *ftsQ*, *ftsL*, and *divIC* (2, 12, 13), $ftsW_{Sc}$ was not essential for growth or viability in *S. coelicolor*. Because *murG* (located 7 nucleotides downstream of $ftsW_{Sc}$) appears to be an essential gene in *S. coelicolor* (P. Bidey and J. R. McCormick, unpublished results), strains containing an unmarked, in-frame deletion of $ftsW_{Sc}$ (Δ*ftsW*) were isolated by transformation using pPB10Δ (pPB10 treated with FLP recombinase) to avoid the potential effects of transcriptional polarity on downstream genes. One representative

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† Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 31 October 2008.

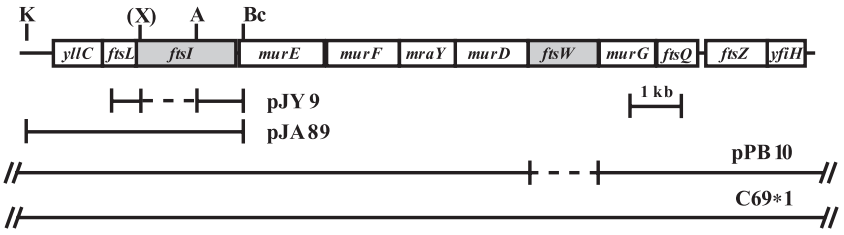

FIG. 1. Chromosomal region containing $ftsI_{Sc}$ and $ftsW_{Sc}$. The *S. coelicolor dcw* cluster containing $ftsI_{Sc}$ and $ftsW_{Sc}$ is shown. Open boxes indicate gene positions. Key restriction sites used to make mutations and complementation plasmids are shown [A, Acc65I; Bc, BclI; K, KpnI; X, XbaI; (X), introduced by PCR]. The diagram below the map contains horizontal bars representing the inserts of plasmids used in the construction of deletion mutations (pJY9 and pPB10) and those used in genetic complementation experiments, pJA89 and C69*1. Deletion-insertion mutations are indicated by the dashed lines (pJY9 and pPB10).

PFB22-derived marker replacement strain that was apramycin sensitive and kanamycin sensitive was named PFB26 (Δ*ftsW*). PFB22 and PFB26 were verified by Southern blot analysis and diagnostic PCR, respectively (see the supplemental material). PFB26 (Δ*ftsW*) grew well, producing colonies that were more robust than those of PFB22 [Δ*ftsW*::*acc*(*3*)*IV*] on all media (MM, R2YE, and MS medium [10]) tested, indicating that the *aac*(*3*)*IV* cassette did have a partial polar effect on the expression of *murG* (and other downstream genes). Colonies of PFB26 produced a slightly less gray pigmented aerial mycelium than the wild type when grown on MS agar (Fig. 2). Consistent with the pale gray aerial mycelium on MS medium, the *ftsW* mutant produced infrequent chains of spores (data not shown). Unlike *ftsZ*- and *ftsQ*-null mutants (12, 13), the $ftsW_{Sc}$-null mutant PFB26 did not overproduce the blue-pigmented antibiotic actinorhodin on MM agar, suggesting that vegetative cross-wall formation is not drastically impaired (data not shown).

Similar to the *ftsL* and *divIC* mutants (2), the strain containing Δ*ftsW* exhibited cell division defects during sporulation, as judged by phase-contrast microscopy. When grown on MM agar containing glucose, the Δ*ftsW* strain displayed a variety of aerial-filament morphologies, including smooth aerial hyphae (10%), aerial hyphae with regularly spaced shallow constrictions (45%), and aerial hyphae containing chains of normal spores, as well as chains of spores with lysed compartments and/or abnormally sized and shaped spores (45%) (Fig. 2E). These morphologies were terminal phenotypes, and hyphae with shallow constrictions were not eventually converted into chains of spores. When PFB26 (Δ*ftsW*) was grown on the osmotically enhanced medium R2YE, the division defect was greatly exacerbated, and the number of aerial filaments observed was reduced. On R2YE, the aerial mycelium contained undifferentiated aerial hyphae (70%) and aerial filaments containing shallow regularly spaced constrictions (30%) (Fig. 2B). The constrictions appeared to be shallower than those produced by *ftsL*- and *divIC*-null strains (2).

TABLE 1. *S. coelicolor* strains used in this study

Strain[a]	Genotype	Reference or source
DU152	Δ*divIC*::*aphI*	2
DU191	Δ*ftsL*::*hyg*	2
HU133	Δ*ftsZ*::*aphI*	13
HU151	Δ*ftsQ*::*addA*	12
J2210	Δ*whiH*::*hyg*	17
JBY5	Δ*ftsI*::*aphI*	This study
M145	Prototroph $SCP1^-$ $SCP2^-$	10
PFB22	Δ*ftsW*::*acc*(*3*)*IV*	This study
PFB26	Δ*ftsW*	This study

[a] All mutant strains are M145 derivatives.

We constructed a pOJ260-derived plasmid (4) called pJY9 by using in vitro recombination and a restriction site introduced by PCR. pJY9 contains a deletion-insertion mutation, in which an internal 1.2-kb fragment of $ftsI_{Sc}$ (codons 10 to 406 of 654) was replaced by *aphI* (Fig. 1). This nonreplicating mutagenic plasmid (pJY9) was introduced by transformation into wild-type *S. coelicolor* strain M145. Independent deletion-insertion mutants were isolated as neomycin-resistant, apramycin-sensitive transformants, and the representative marker replacement strain JBY5 (Δ*ftsI*::*aphI*) was used for further study. JBY5 was verified by Southern blot analysis (see the supplemental material). Thus, as with every other division mutant characterized to date, $ftsI_{Sc}$ is dispensable for growth and viability of *S. coelicolor*, making this an advantageous system for studying bacterial cytokinesis. We did not anticipate polar effects of the Δ*ftsI*::*aphI* mutation due to our analysis of a similar *ftsL* mutant (2). *aphI* was inserted in the same orientation as $ftsI_{Sc}$, and a gap between $ftsI_{Sc}$ and *murE* suggests the potential for a promoter in the intercistronic space (208 nucleotides). JBY5 grew well and produced robust colonies on MM, R2YE, and MS media, and its aerial mycelium was paler gray on MS medium than that of the wild type (Fig. 2). Consistent with the pale-gray aerial mycelium on MS medium, the *ftsI* mutant produced infrequent chains of spores (data not shown) and the vegetative hyphae occasionally contained regions with bulges. Unlike the *ftsZ*- and *ftsQ*-null mutants, the $ftsI_{Sc}$-null mutant did not overproduce the blue-pigmented antibiotic actinorhodin on MM agar, suggesting that vegetative cross-wall formation is not drastically impaired (data not shown).

When grown on MM, the $ftsI_{Sc}$-null strain was essentially indistinguishable from the $ftsW_{Sc}$-null strain, displaying chains of spores (approximately 45% of which was a mixture of normal and aberrant sizes and shapes), as well as aerial filaments with regularly spaced, shallow constrictions (45%) and those completely devoid of constrictions (10%) (Fig. 2F). These morphologies were terminal phenotypes, and hyphae with shallow constrictions were not eventually converted into chains of spores. However, when the strain was grown on the osmotically enhanced medium R2YE, the division defect was greatly exacerbated, and the number of aerial filaments observed was

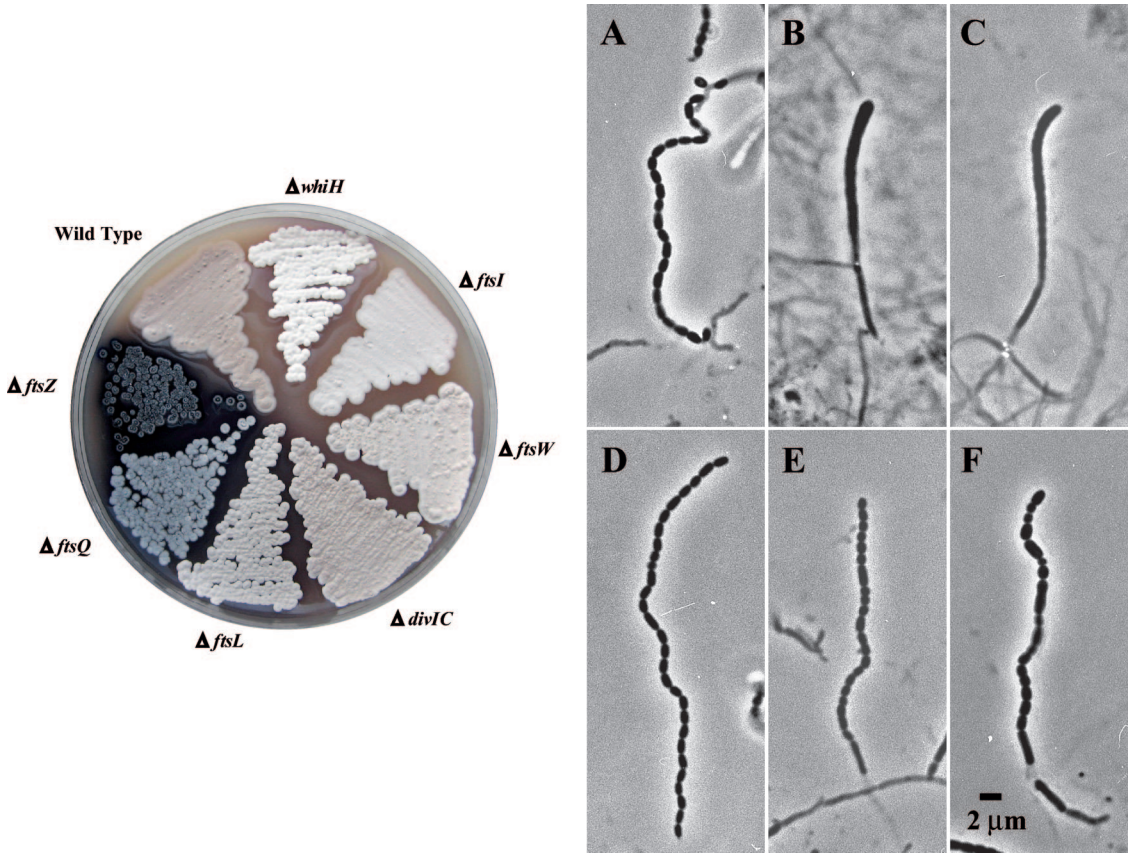

FIG. 2. Growth phenotypes of mutants and phase-contrast microscopy of wild-type and mutant aerial hyphae. (Left) MS agar medium was used to visualize the gray pigment associated with spore formation within the aerial mycelium (colony surface). Cultures were incubated for 5 days at 30°C. Strains M145 (wild type), HU133 (Δ*ftsZ*), HU151 (Δ*ftsQ*), DU152 (Δ*divIC*), DU191 (Δ*ftsL*), JBY5 (Δ*ftsI*), PFB26 (Δ*ftsW*), and J2210 (Δ*whiH*, white mutant control) are shown (see Table 1). (Right) Phase-contrast micrographs of coverslip lifts removed after 6 days of growth on R2YE (A to C) or glucose MM (D to F) are shown. Panels A and D show wild-type spore chains (strain M145). Also shown are aerial hyphae of the $ftsW_{Sc}$-null mutant PFB26 (B, R2YE; E, MM) and $ftsI_{Sc}$-null mutant JBY5 (C, R2YE; F, MM). The sporulation phenotype is heterogeneous, and only one category of aerial hyphae produced by the division mutants is shown (see text for details).

reduced. The phenotype of the $ftsI_{Sc}$-null mutant was characterized by short, cane-shaped aerial filaments possessing very shallow, regularly spaced constrictions (30%) (Fig. 2C) and undifferentiated aerial hyphae (70%).

Similar to the *ftsL* and *divIC* mutants (2), the $ftsI_{Sc}$ and $ftsW_{Sc}$ mutants were more defective when grown on the osmotically enhanced medium R2YE. To determine if the same parameter affected the division phenotypes of the $ftsI_{Sc}$ and $ftsW_{Sc}$ mutants, we omitted sucrose from R2YE (normal concentration, 0.3 M). We found that the $ftsI_{Sc}$- and $ftsW_{Sc}$-null mutants grown on R2YE without sucrose mimicked the phenotypes of the mutants grown on MM (data not shown) (1). Interestingly, the *ftsI* mutant produced aerial filaments that were shorter than those produced by the *ftsW*, *ftsL*, and *divIC* mutants on the osmotically enhanced medium (data not shown). We speculate that FtsI may also be involved in peptidoglycan synthesis during elongation of the lateral walls of aerial hyphae, resulting in shortened cane-shaped filaments produced by the mutant.

Finally, because a significant fraction of the aerial hyphae of the $ftsI_{Sc}$ and $ftsW_{Sc}$ mutants develop into chains of separated spores on MM, we infer that functional FtsZ rings form, producing evenly spaced sporulation septa under certain conditions. This is in contrast to *Mycobacterium smegmatis*, in which it has been shown that FtsZ localized to the midcell when FtsW was depleted but cell separation was not completed (6).

Genetic complementation experiments. Complementation experiments were performed with the most restrictive medium (osmotically enhanced R2YE) to ensure that the division defects observed were imparted by the introduced mutations. pJA89 (2), a low-copy-number plasmid containing P_{dcw}-*yllC-ftsL-*$ftsI_{Sc}$ (Fig. 1), partially restored sporulation to the $ftsI_{Sc}$-null mutant, indicating that the phenotype is due in part to the absence of $ftsI_{Sc}$ (data not shown) (1). C69*1 (a matable version of cosmid C69) (Fig. 1), which contains the *dcw* cluster and flanking regions, was able to complement the division defect imparted by the $ftsW_{Sc}$-null mutation when integrated by homologous recombination (data not shown) (1). We conclude that the division phenotype was linked to the Δ*ftsW* mutation.

Septal morphologies of the $ftsI_{Sc}$- and $ftsW_{Sc}$-null mutants. We examined the morphology of the septa in hyphae by transmission electron microscopy (TEM) as previously described (2), using strains grown on R2YE, where the division defect was most severe. Approximately half of all vegetative cross-

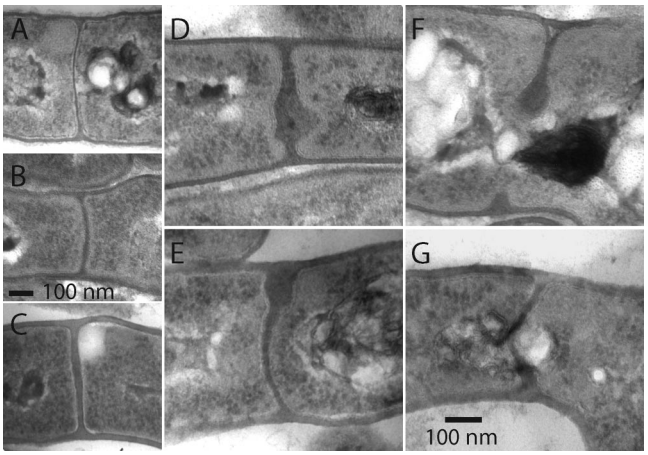

FIG. 3. TEMs showing vegetative cross-walls in wild-type and division mutant filaments. Strains were grown for 6 days on R2YE, the medium showing the largest defect in division, and then prepared for TEM examination. (A) A wild-type vegetative cross-wall (strain M145), which can vary in thickness, is shown. (B and C) Normal vegetative cross-walls (50%) are shown for Δ*ftsI*$_{Sc}$ (JBY5) and Δ*ftsW*$_{Sc}$ (PFB26) mutants, respectively. (D to G) The other half of the cross-walls are formed aberrantly, as shown for the *ftsI*$_{Sc}$-null mutant (D and F) and the for *ftsW*$_{Sc}$-null mutant (E and G).

walls examined in the *ftsI*$_{Sc}$ and *ftsW*$_{Sc}$ mutants appeared to be normal (Fig. 3, compare panels A to C). Otherwise, the cross-walls in the mutants were aberrant, with cell wall material on both sides of the division site that had not met in the middle (Fig. 3F), or completed cross-walls were wavy and/or thickened (Fig. 3D, E, and G). Complete cross-walls were reported in a concurrent study of *ftsI* and *ftsW* mutants (14). However, those observations were done with cells grown on a very unusual choice of medium for *Streptomyces* (2× yeast-tryptone [YT] medium), and perhaps the salt concentration may have affected the observed phenotypes. Unexpectedly, no sporulation septa could be definitively identified for either mutant. The surfaces of *ftsW*$_{Sc}$ and *ftsI*$_{Sc}$ mutant colonies were difficult to embed for TEM, and the sporadic population of aerial filaments observed by phase-contrast microscopy may have been too infrequent in the TEM sections. The mutants produced mostly aerial hyphae that lacked constrictions, and constrictions are used to unambiguously identify aerial hyphae.

Summary and conclusions. Of the six division genes of *S. coelicolor* genetically analyzed to date (2, 12–14; this study), only two are more strictly required for division under most circumstances (*ftsQ* and *ftsZ*) and only one is absolutely required (*ftsZ*). By analogy to the divisome described for other systems, it appears that proteins presumably recruited late in the division pathway of *S. coelicolor* are not absolutely required for cell division under certain conditions. Because *S. coelicolor* is predicted to encode four SEDS/PBP pairs, one other pair must be able to substitute for FtsW/FtsI to support cell division under some growth conditions. One of the SEDS proteins presumably moves lipid II-linked precursors across the membrane (19), and a PBP must synthesize septal peptidoglycan.

Nucleotide sequence accession numbers. The sequences of *ftsI*$_{Sc}$ and *ftsW*$_{Sc}$ were deposited in GenBank under accession numbers AF123319 and U10879, respectively. Subsequently, the sequences determined by the genome project agreed (3).

We thank Joseph Suhan (Carnegie Mellon University) for providing assistance with electron microscopy and Berthold Gust for advice concerning lambda Red recombination.

This work was supported by grant GM56915 from the National Institutes of Health (to J.R.M.) and an award from Beta Beta Beta National Biology Honor Society (to A.B.C.).

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