

# Cell Division by Longitudinal Scission in the Insect Endosymbiont *Spiroplasma poulsonii*

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**ABSTRACT** *Spiroplasma* bacteria are highly motile bacteria with no cell wall and a helical morphology. This clade includes many vertically transmitted insect endosymbionts, including *Spiroplasma poulsonii*, a natural endosymbiont of *Drosophila melanogaster*. *S. poulsonii* bacteria are mainly found in the hemolymph of infected female flies and exhibit efficient vertical transmission from mother to offspring. As is the case for many facultative endosymbionts, *S. poulsonii* can manipulate the reproduction of its host; in particular, *S. poulsonii* induces male killing in *Drosophila melanogaster*. Here, we analyze the morphology of *S. poulsonii* obtained from the hemolymph of infected *Drosophila*. This endosymbiont was not only found as long helical filaments, as previously described, but was also found in a Y-shaped form. The use of electron microscopy, immunogold staining of the FtsZ protein, and antibiotic treatment unambiguously linked the Y shape of *S. poulsonii* to cell division. Observation of the Y shape in another *Spiroplasma*, *S. citri*, and anecdotic observations from the literature suggest that cell division by longitudinal scission might be prevalent in the *Spiroplasma* clade. Our study is the first to report the Y-shape mode of cell division in an endosymbiotic bacterium and adds *Spiroplasma* to the so far limited group of bacteria known to utilize this cell division mode.

**IMPORTANCE** Most bacteria rely on binary fission, which involves elongation of the bacteria and DNA replication, followed by splitting into two parts. Examples of bacteria with a Y-shape longitudinal scission remain scarce. Here, we report that *Spiroplasma poulsonii*, an endosymbiotic bacterium living inside the fruit fly *Drosophila melanogaster*, divide with the longitudinal mode of cell division. Observations of the Y shape in another *Spiroplasma*, *S. citri*, suggest that this mode of scission might be prevalent in the *Spiroplasma* clade. *Spiroplasma* bacteria are wall-less bacteria with a distinctive helical shape, and these bacteria are always associated with arthropods, notably insects. Our study raises the hypothesis that this mode of cell division by longitudinal scission could be linked to the symbiotic mode of life of these bacteria.

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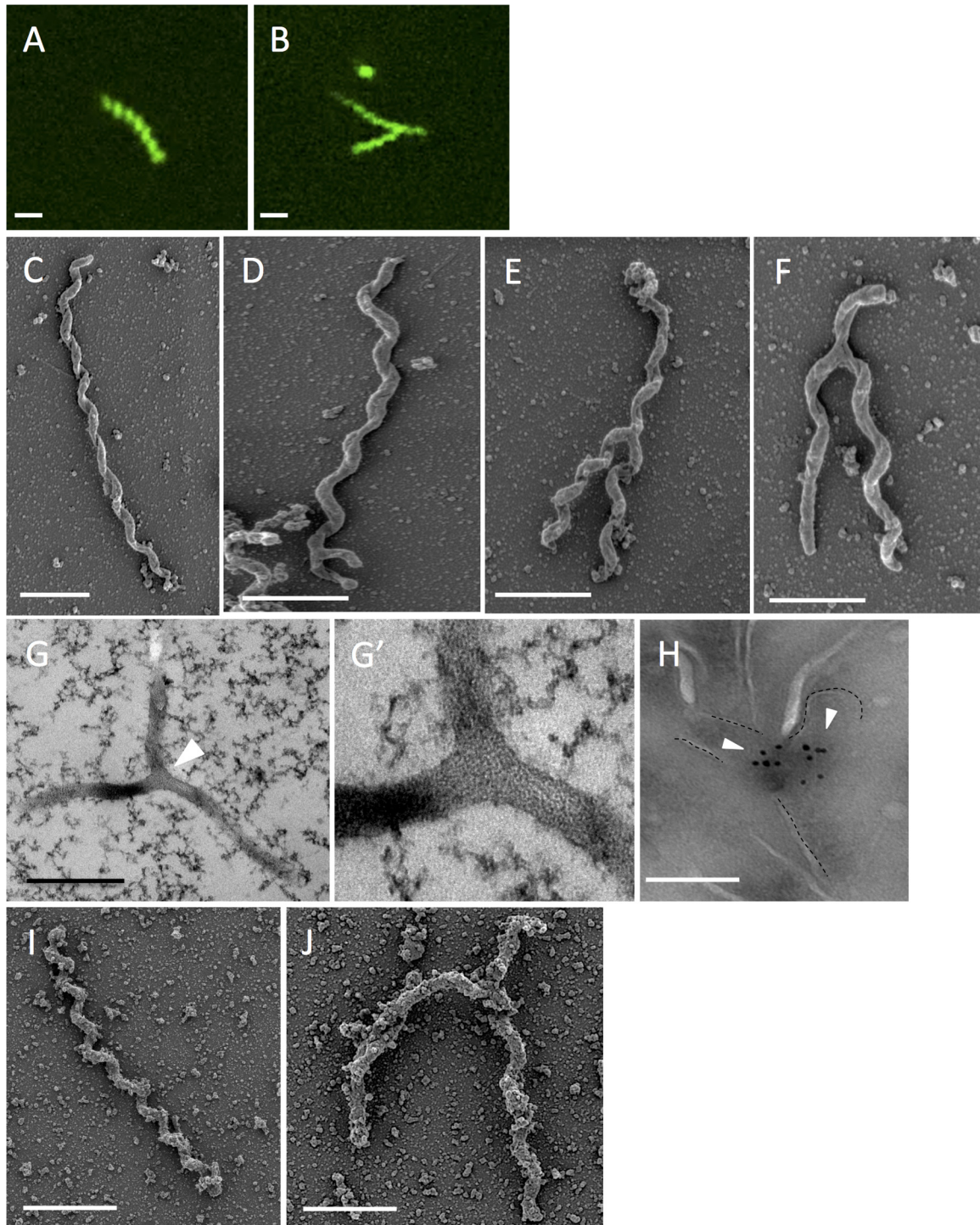
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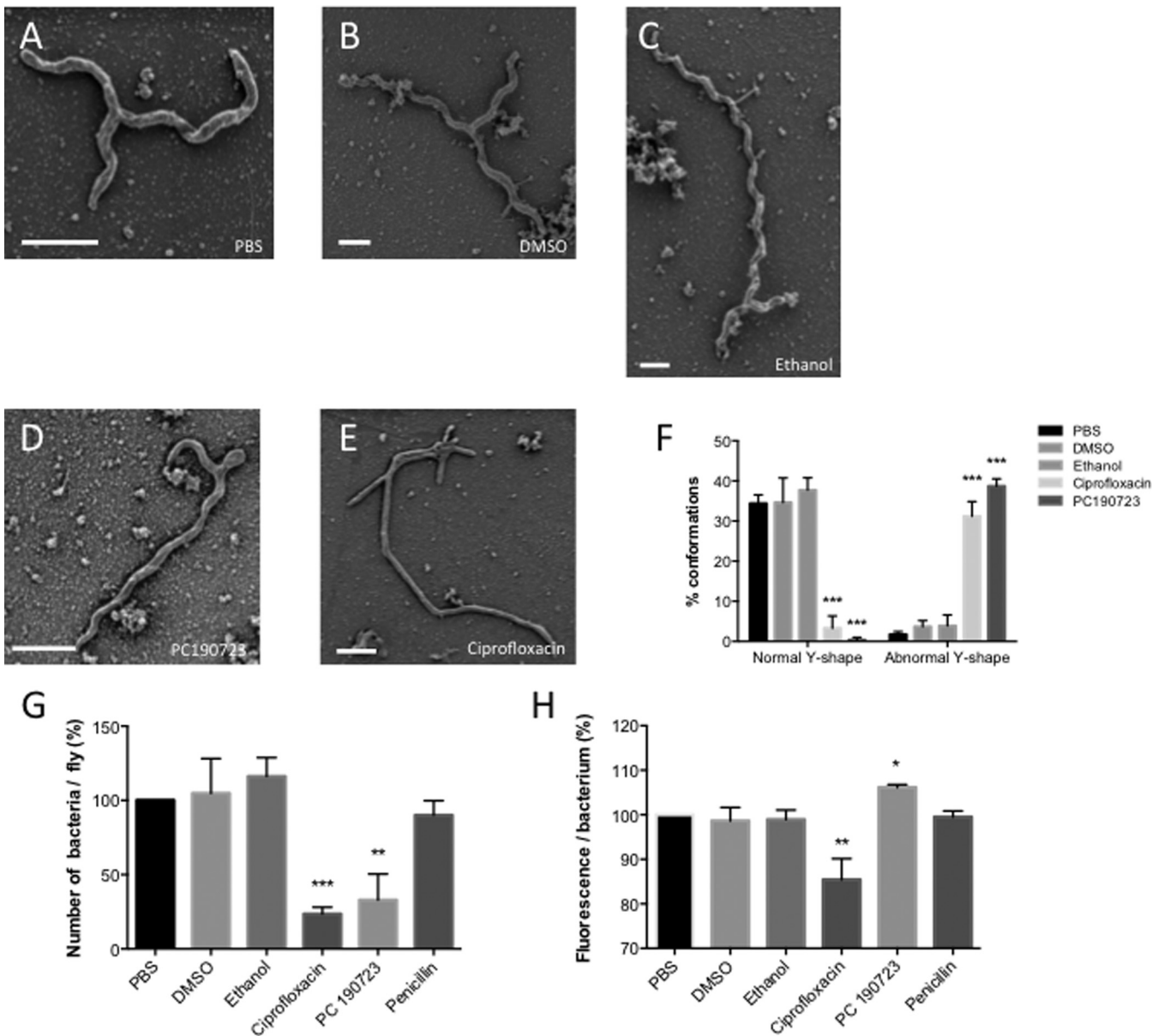
*Spiroplasma* bacteria are members of the *Mollicutes* class, a wall-less eubacterial group related to Gram-positive bacteria (1). These bacteria exhibit a distinctive helical shape and high motility. *Spiroplasma* bacteria are widely associated with arthropods and found in 5 to 10% of all insect species. While some species are horizontally transmitted insect pathogens or commensals in the gut, other lineages exhibit transovarial vertical transmission from mother to offspring. *Spiroplasma poulsonii* is one of the species exhibiting vertical transmission, and together with *Wolbachia*, are two natural endosymbionts known from *Drosophila melanogaster*.

*S. poulsonii* bacteria are primarily found in the hemolymph of adult flies, where they are neither detected nor affected by the *Drosophila* immune system. The proliferation of *Spiroplasma* is constrained by the availability of hemolymph lipids (2, 3). *S. poulsonii* uses the yolk uptake machinery to colonize the germ line during oogenesis to ensure vertical transmission (4). Similar to other facultative endosymbionts, *S. poulsonii* can affect the sex ratio of its host by inducing male killing (causing death of male embryos at early time points) (5). It has been proposed that reproductive manipulation and the ability of *Spiroplasma* to protect

*Drosophila* against certain parasites are among the main driving forces that maintain this facultative endosymbiont in fly populations (6). There have been a number of studies recently investigating how *Spiroplasma* impacts its insect host; however, little is known about the biology of *S. poulsonii* itself. This led us to investigate the morphology of *S. poulsonii* (strain MSRO; for experimental methods, see Text S1 in the supplemental material) derived from fly hemolymph extracts. We chose 1- to 2-week-old females, since this time period corresponds to the exponential growth phase of this endosymbiont (2). Use of the nucleic acid stain SYTO9 reveals the presence of *Spiroplasma* with a classical helical and linear morphology (Fig. 1A). Surprisingly, up to 30% of bacteria show a Y-shape morphology, which has not been previously characterized (Fig. 1B). Scanning electron microscopy (SEM) of hemolymph samples from *Spiroplasma*-infected *D. melanogaster* females also confirms the presence of Y-shaped *Spiroplasma*. Typical linear bacteria have 5 to 15 helices, with the length of the bacteria ranging from 0.8  $\mu\text{m}$  to 5  $\mu\text{m}$  and the width ranging from 50 to 100 nm (Fig. 1C). SEM confirmed the presence of Y-shaped bacteria with one main branch of various sizes, split-



**FIG 1** Presence of Y-shaped *S. poulsonii* in *Drosophila* hemolymph samples. (A and B) Fluorescence microscopy images showing SYTO9-stained *S. poulsonii* from freshly extracted hemolymph from 1-week-old *Drosophila melanogaster* flies. Bars, 1  $\mu\text{m}$ . (C to F) SEM of *S. poulsonii* extracted from 1-week-old *Spiroplasma*-infected female flies. *S. poulsonii* can be found as one elongated body (C) or with a Y-shape conformation (D to F) with variation in the length of the arms. Bars, 1  $\mu\text{m}$ . (G and zoom in G') TEM of *S. poulsonii* from freshly extracted *Drosophila* hemolymph. The white arrowhead shows the branching. Bar, 1  $\mu\text{m}$ . (H) Immunogold labeling pattern of the FtsZ protein with an anti-FtsZ antibody. Y-shaped bacteria have two FtsZ protein aggregates. Bar, 200 nm. (I and J) SEM of *in vitro*-cultured *S. citri*. *S. citri* can be found as one elongated body (I) or with a Y-shaped conformation (J). Bars, 1  $\mu\text{m}$ . The presence of aggregates might be due to protein enrichment in the medium used to cultivate *S. citri*.



**FIG 2** Impacts of various antibiotics on the Y-shaped form of *Spiroplasma*. (A to E) SEM of *S. poulsonii* extracted from *Spiroplasma*-infected female flies injected with phosphate-buffered saline (PBS) (A), DMSO (B), ethanol (C), PC190723 (D), or ciprofloxacin (E). One-week-old females were injected with molecules, and hemolymph was extracted after 4 days. Bars, 500 nm. (F) Quantification of normal and abnormal Y-shape *S. poulsonii* by SEM in hemolymph samples collected from treated flies. \*\*\*,  $P < 0.005$ . Values are means plus standard deviations (SD) (error bars) of data pooled from three independent experiments with 100 bacteria for each count. Twenty flies were used to extract fresh hemolymph for each experiment. (G) Evaluation of bacterial count per fly following each treatment. *Spiroplasma* bacteria were counted 1 week after injection. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.005$ . Values are means plus SD of data pooled from three independent experiments with 20 flies for each count. (H) Quantification of the amount of fluorescence per bacteria. DNA fluorescence per *Spiroplasma* was measured 4 days after injection. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . Values are means plus SD of data pooled from three independent experiments with 20 flies tested for each count.

ting into two thinner arms of identical length (Fig. 1D, E, and F). To exclude the possibility that the Y-shaped form results from the twisting of two bacteria, we performed high-pressure freezing followed by transmission electron microscopy (TEM) on hemolymph samples. Our results confirm that the Y shapes correspond to one bacterium with cytoplasmic continuity between the two arms and the main trunk (Fig. 1G, white arrowhead; high-magnification view shown in Fig. 1G').

Cytokinesis is the process by which the cytoplasm of one cell is

divided in two cells. In bacteria, it involves the guanosine triphosphatase (GTPase) FtsZ (filamenting temperature-sensitive Z), which assembles at the inner face of the bacterial membrane to form a Z-ring, which subsequently recruits molecules to provide the contractile force for membrane invagination (7, 8). One gene encoding a FtsZ protein is present in the *S. poulsonii* genome (9). To assess whether the *S. poulsonii* Y shape is indeed linked to cell division, we performed immunogold labeling against FtsZ protein using a commercially available antibody. Figure 1H shows accu-



mulation of signals corresponding to FtsZ in the branching area of Y-shape bacteria, with gold particles forming two small aggregates at the beginning of the two arms (white arrowheads). No gold particle aggregate was observed in linear bacteria.

To confirm our conclusion, we analyzed the impacts of antibiotics that affect cell division on *S. poulsonii* morphology. Antibiotics were directly injected into the body cavities of *Spiroplasma*-infected flies, and hemolymph samples were collected 4 days later for observations and 1 week later for bacterial quantification in flies. Injection of PC190723 (dissolved in dimethyl sulfoxide [DMSO]), a cell division inhibitor known to block the GTPase activity of FtsZ leads to aberrant Y-shape morphology with an aborted arm situated at one extremity of the main trunk (Fig. 2D; quantified in Fig. 2F). Injection of ciprofloxacin (dissolved in ethanol), a fluoroquinolone antibiotic which inhibits DNA gyrase activity, and therefore DNA replication, results in abnormal shaped *Spiroplasma* with no spiral and multibranching with 3 to 5 arms of various sizes linked to the main trunk (Fig. 2E; quantified in Fig. 2F). We speculate that this morphology results from multiple scission departures that aborted precociously. This also suggests that DNA replication is not mandatory for the initiation of branching. Injection of DMSO or ethanol, the two antibiotic solvents, or penicillin, an antibiotic that targets peptidoglycan, a cell wall component absent in *Spiroplasma*, did not affect *S. poulsonii* morphology, indicating that the effects are caused by the two molecules (Fig. 2A to C). Figure 2G shows that PC190723 and ciprofloxacin antibiotic that affect *Spiroplasma* morphology also reduce bacterial growth as measured by quantitative PCR (qPCR).

SYTO9 DNA staining demonstrates the presence of DNA all along the arms of both linear and Y-shaped *Spiroplasma* (Fig. 1A and B). To investigate the link between DNA synthesis and *Spiroplasma* morphology, we analyze by qPCR the presence of DNA in bacteria treated with PC190723 and ciprofloxacin. Remarkably, PC190723-treated bacteria presented more fluorescence, which is consistent with the notion that FtsZ target drug inhibits cell division, but not DNA replication. In contrast, a lower DNA signal was observed upon treatment with the DNA synthesis inhibitor ciprofloxacin. Taken together, our data suggest that the production of a new arm occurs before replication of DNA but that the final scission of the two cells required DNA synthesis completion. Unfortunately, due to the high motility of *S. poulsonii* and their quick adherence (within minutes) to the substratum, we were unable to perform live imaging of *S. poulsonii* division.

Most bacteria rely on binary fission, which involved elongation of the bacteria and DNA replication before splitting into two parts. Examples of bacteria with a Y-shape division remain scarce. Bacteria with Y-shape division include *Actinomyces* (10) and *Bifidobacterium* (11). It is interesting to connect our observation to a recent study revealing the presence of the Y shape in another symbiotic bacterium. Leisch et al. have shown that the gammaproteobacterium ectosymbiont, which attaches to the surface of the marine nematode *Laxus oneistus*, grows laterally and divides longitudinally (13). This mode of cell division is thought to be important for maintaining the attachment of both daughter cells to the nematode surface (12). In contrast to *Spiroplasma*, longitudinal scission takes place simultaneously on both extremities of the ectosymbiont. Y-shaped bacteria have also been observed in another symbiotic context that involves nitrogen-fixing *Rhizobium* and leguminous plants. Y-shaped *Rhizobium* bacteria are

abundant in the nitrogen fixation zone of nodules, suggesting that this morphology could be induced by the plant host (14).

Our report adds *S. poulsonii* to the short list of bacteria with a longitudinal mode of cell division and is the first to show this feature in an insect endosymbiont. Screening of the literature reveals previous observations of branched morphologies in tick-associated *Spiroplasma ixodetis* (15), plant-associated *Spiroplasma citri* (16), and an unidentified *Spiroplasma* subspecies found in the Chinese mitten crab *Eriocheir sinensis* (17). However, Garnier et al. proposed that *Spiroplasma citri* divides by elongating before constricting to give two daughter cells (18). Careful analysis of *S. citri* cultures reveals the presence of the Y shape (Fig. 1I and J), suggesting that this mode of division could be rather prevalent in the *Spiroplasma* clade. An open question is to determine the nature of the selective pressure that leads to this mode of cell division. The prevalence of the Y shape in bacteria establishing symbiotic interactions with eukaryotes may allow the coupling of longitudinal fission with signals from the host, as proposed for *Rhizobium* or the nematode ectosymbionts. Alternatively, this mode of septation might be related to the motility of *Spiroplasma*, which utilizes long contractile fibers that run from one end of the cell to the other (19). Indeed, we can speculate that division begins at one extremity of the bacteria, following by longitudinal opening (“zip-progression”) of the cell, which could explain the presence of a main trunk and two arms of the same length, before the separation of the two daughter cells on the other extremity. A longitudinal scission would allow the preservation of the fiber network such that fibers are not cut in half. Future studies should search for the presence of other Y-shaped bacteria in other microbial symbioses and characterize their functional relevance.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00881-16/-/DCSupplemental>.

Text S1, PDF file, 0.1 MB.

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