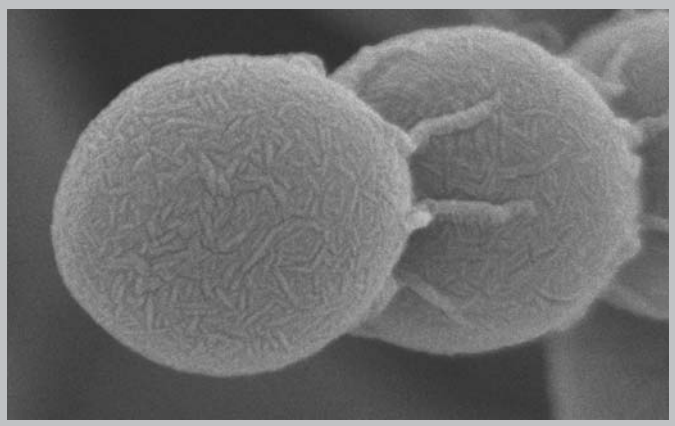
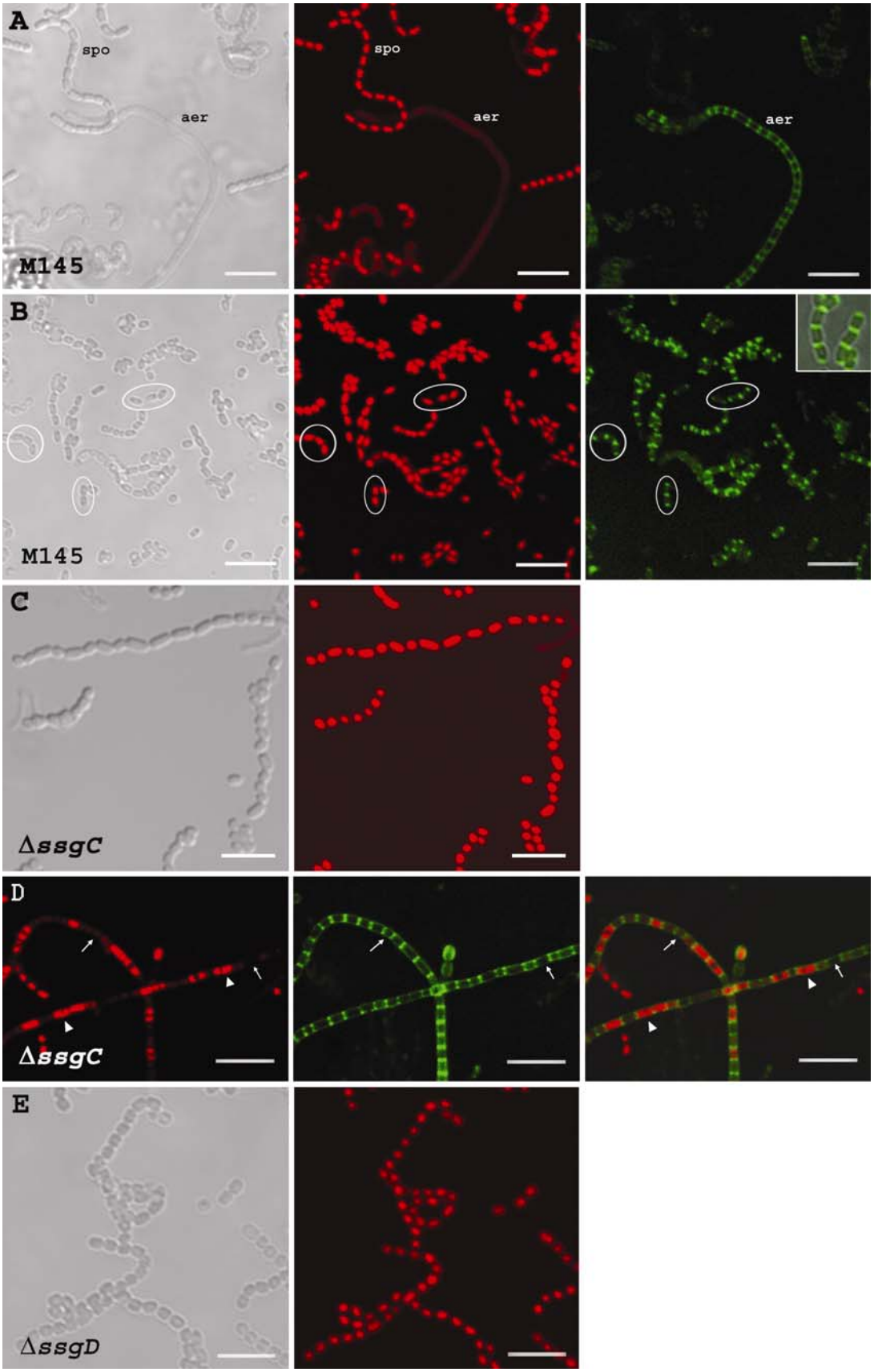
# Control of sporulation-specific cell division in Streptomyces coelicolor

Noens, E.

# Appendices

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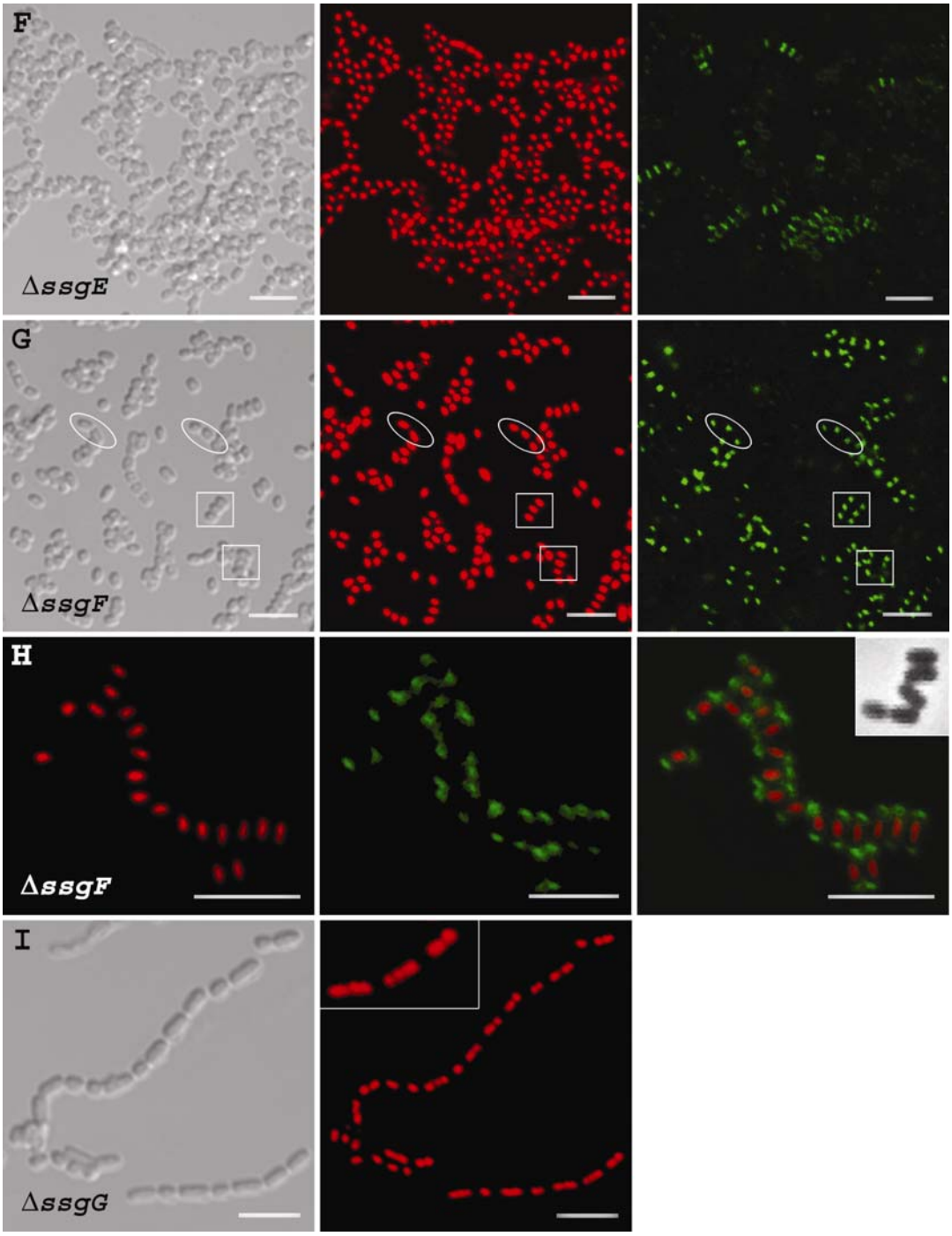


**Figure 5, Chapter 2: Analysis of the *ssgC-G* mutants by confocal fluorescence microscopy.**
Samples were prepared from surface-grown cultures of the parental strain *S. coelicolor* M145 **(A)** and its mutant derivatives Δ*ssgC* **(C-D)**, Δ*ssgD* **(E)**, Δ*ssgE* **(F)**, Δ*ssgF* **(G-H)**, and Δ*ssgG* **(I)** all grown on SFM plates for 6 days at 30°C. *S. coelicolor* M145 **(B)** was grown on SFM plates for 2-4 days at 30°C. DNA and peptidoglycan subunits were visualised with PI (red) and fluorescein-WGA (green), respectively. The first column shows light microscopy micrographs, the middle column shows DNA, and the third column shows peptidoglycan subunits **(A-C, E-G, I)** or the first column shows DNA, the second shows peptidoglycan subunits and the third shows an overlay of PI and WGA **(D-H)**. **(B, insert)** overlay of fluo-WGA and light microscopy of spores of M145 after 2-4 days of growth, clearly showing staining of spore poles by WGA. **(H, insert)** shows a light microscopy micrograph of Δ*ssgF* spores. **(I, insert)** shows a higher magnification of Δ*ssgG* spores with four and respectively three copies of the chromosome. For mature *ssgD* and *ssgG* mutants no WGA stained septa were detected, and images were therefore omitted. Arrowheads show compartments with multiple chromosomes, small arrows show compartments without DNA, white circles highlight WGA-stained spore poles and squares highlight 'rotated' spores. Bar = 5 μm.

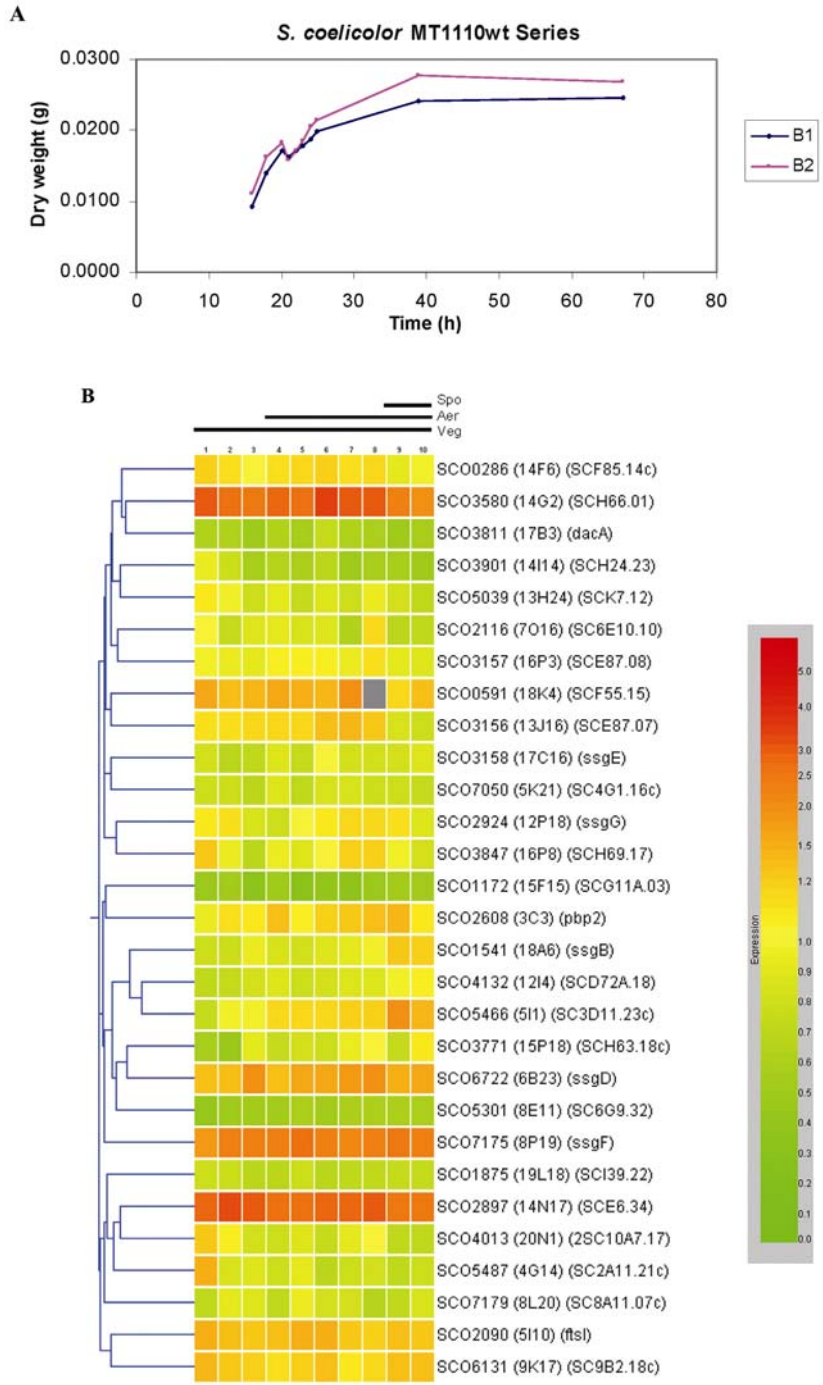


**Figure 7, Chapter 2: DNA microarray analysis of developmental and cell wall-related genes in *S. coelicolor* MT1110.** cDNA from *S. coelicolor* MT1110 (wild type) labelled with Cy3 d-CTP and gDNA from *S. coelicolor* M145 labelled with Cy5 d-CTP were co-hybridised on microarrays. Colour coding: red indicates a high transcriptional level and green indicates a low transcriptional level. Grey represents a data point below a confidence threshold. The same microarrays were used to analyse RNA isolated from *S. lividans* 1326. This resulted in highly similar data for the SALP genes and the most prominent PBPs and autolysins (not shown). **6A.** Growth curves of two biologically independent experiments. Samples were harvested at ten time points (indicated by a dot). Samples: **1-3,** vegetative growth; **4,** transition from vegetative to aerial; **5-8,** aerial growth; **9-10,** sporulation. **6B.** Gene tree of expression profiles of *ssgA*-like genes and genes encoding penicillin-binding proteins (PBPs) and autolysins. RNA time points: (1) 16 h; (2) 18 h; (3) 20 h; (4) 21 h; (5) 22 h; (6) 23 h; (7) 24 h; (8) 25 h; (9) 39 h; (10) 67 h. Sample 4 corresponded to the onset of aerial mycelium formation and spores were already produced at sample 9. **6C.** Colour scale indicating normalised expression levels.

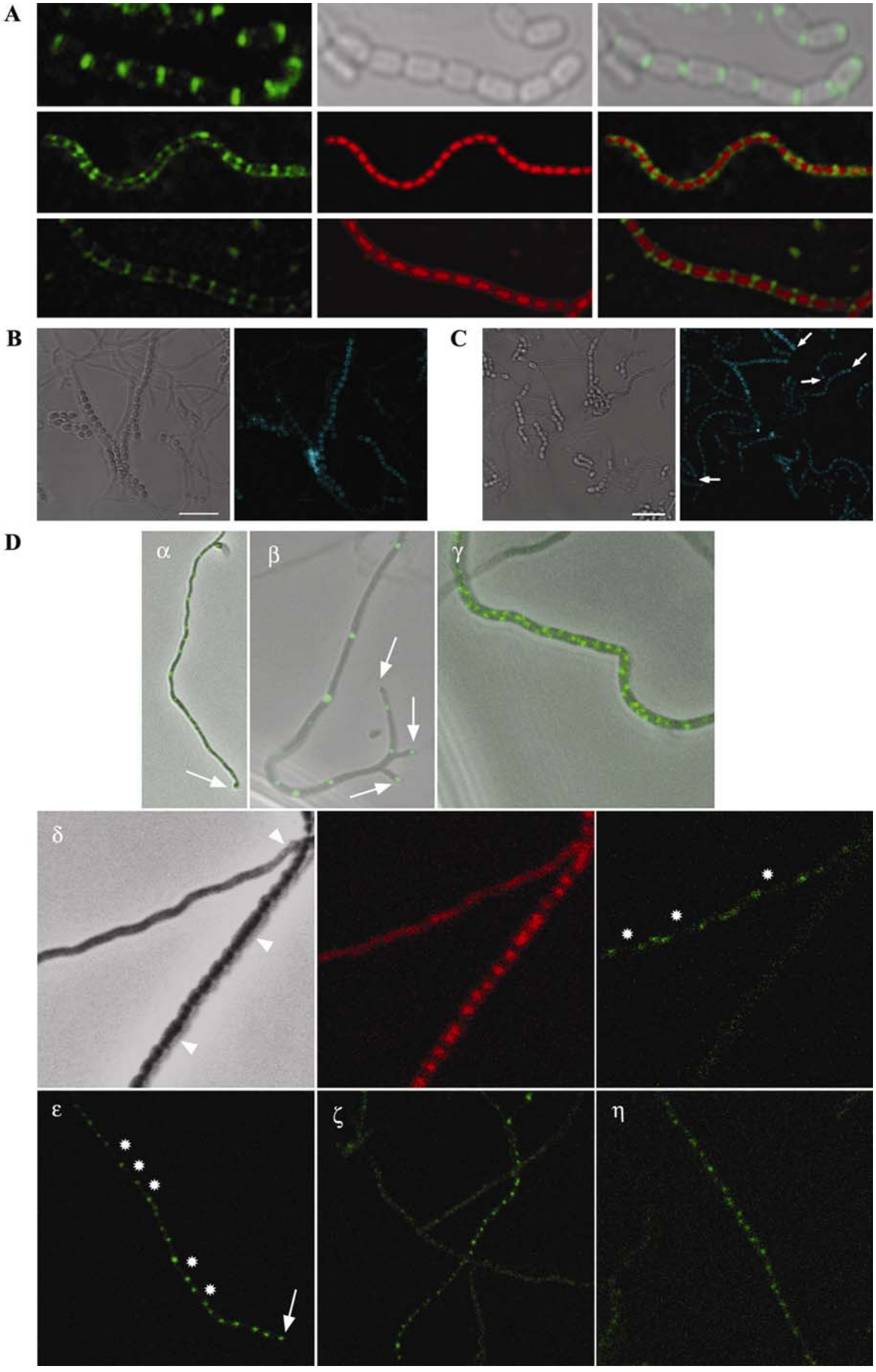
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**Figure 2, Chapter 3, p177: Localisation of SsgB, ssgE, ssgF and SsgG.** Strains were grown on SFM for 5 days (SsgB, SsgE-ECFP and SsgF-ECFP) or 1-4 days (SsgG-EGFP) at 30ºC. DNA was visualised with PI (red) **A.** Localisation of SsgB. The first column shows immuno-fluorescence micographs using fluorescein-conjugated anti-SsgB antibodies, the middle column shows light micrographs (top) and DNA (middle, bottom) and the third column shows overlay images from the left and the middle images. Bar = 2 μm. **B-C.** Non-specific localisation of SsgE-ECFP (B) and ECFP-SsgF (C). ECFP-SsgF shows occasionally brighter foci at the tip of the spores (arrow). Bar = 5 μm. **D.** Localisation of SsgG-EGFP in vegetative hyphae (α-β) and in aerial hyphae (γ-η). α-γ show overlays from light microscopy images and SsgG-EGFP. δ shows light microscopy (left), DNA (middle) and SsgG-EGFP (right) while ε, ζ, η show only SsgG-EGFP. In aerial hyphae, class 1 (γ) shows a staggered pattern while in class 2 (δ-η), foci are laid down in regular pattern, with distances resembling the size between sporulation septa. SsgG was not localised in the spores (δ). Stars show the place where the distance between the foci is double the normal distance and subsequently, spores two times the normal size are created (arrowheads). SsgG-EGFP appeared in the hyphal tips of both vegetative and aerial hyphae (arrows). Bar = 2 μm.

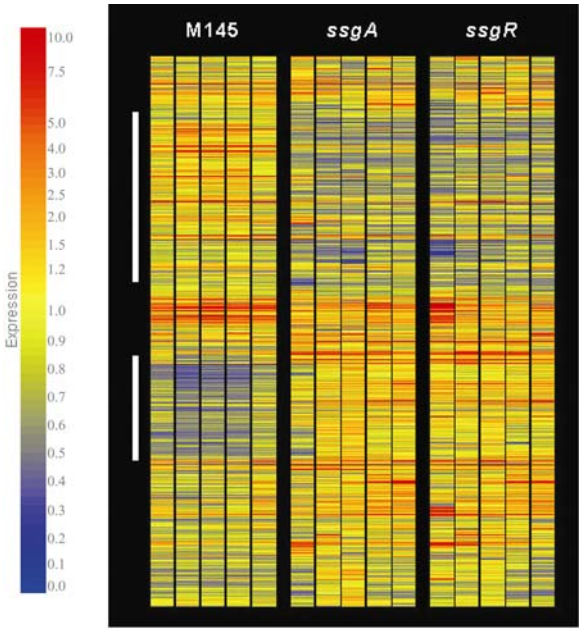


**Figure 4, Chapter 4: Comparison of the global expression patterns of genes in *ssgA* and *ssgR* mutant.** RNA from GSA3 (M145 Δ*ssgA*) and GSR1 (M145 Δ*ssgR*) was isolated from mycelium grown on MM agar, corresponding to vegetative growth (24h), aerial growth (36h, 48h) and sporulation (60h, 72h). Genes were clustered hierachically according to similarity in expression profile. The lanes represent the time points of RNA isolation for the parental strain M145 (left), the *ssgA* mutant (middle), and the *ssgR* mutant (right).

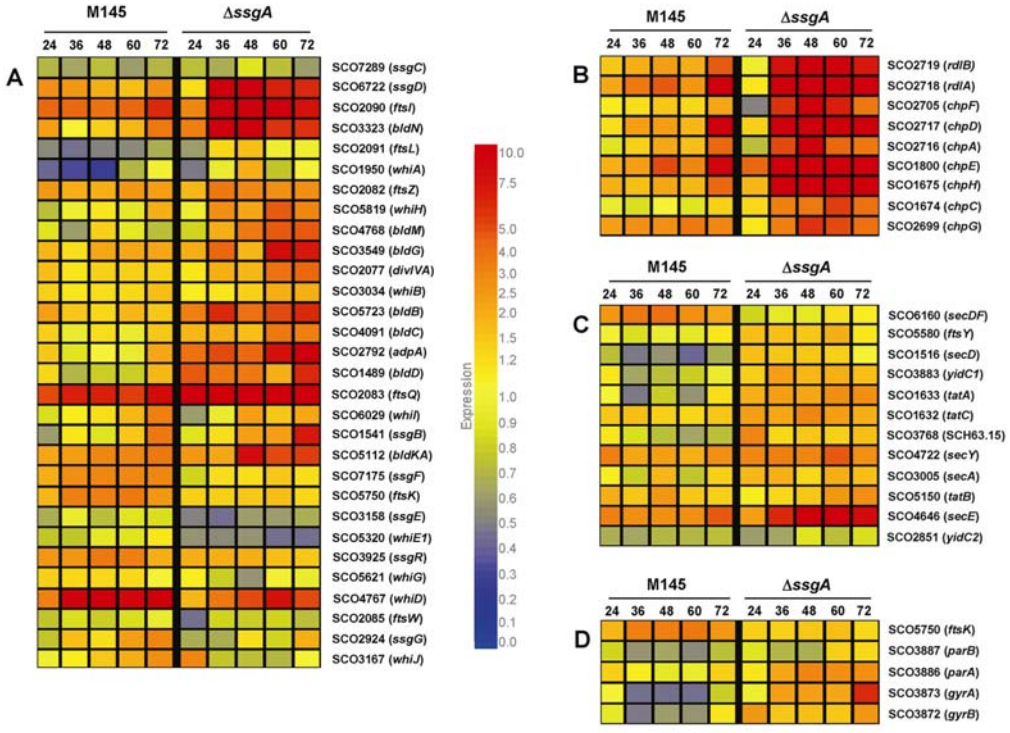


**Figure 5, Chapter 4: Comparison of the expression profiles of several classes of genes between M145 and the *ssgA* mutant.** RNA from M145 and GSA3 (M145 Δ*ssgA*) was isolated from mycelium grown on MM agar, corresponding to vegetative growth (24h), aerial growth (36h, 48h) and sporulation (60h, 72h). Expression profiles of genes are shown, involved in cell division and development (**A**), chaplins and rodlins (**B**), secretion (**C**) and DNA replication and segregation (**D**). Genes were clustered hierarchically according to similarity in expression profile. The lanes represent the time points of RNA isolation for the parental strain M145 (left) and the *ssgA* mutant (right).

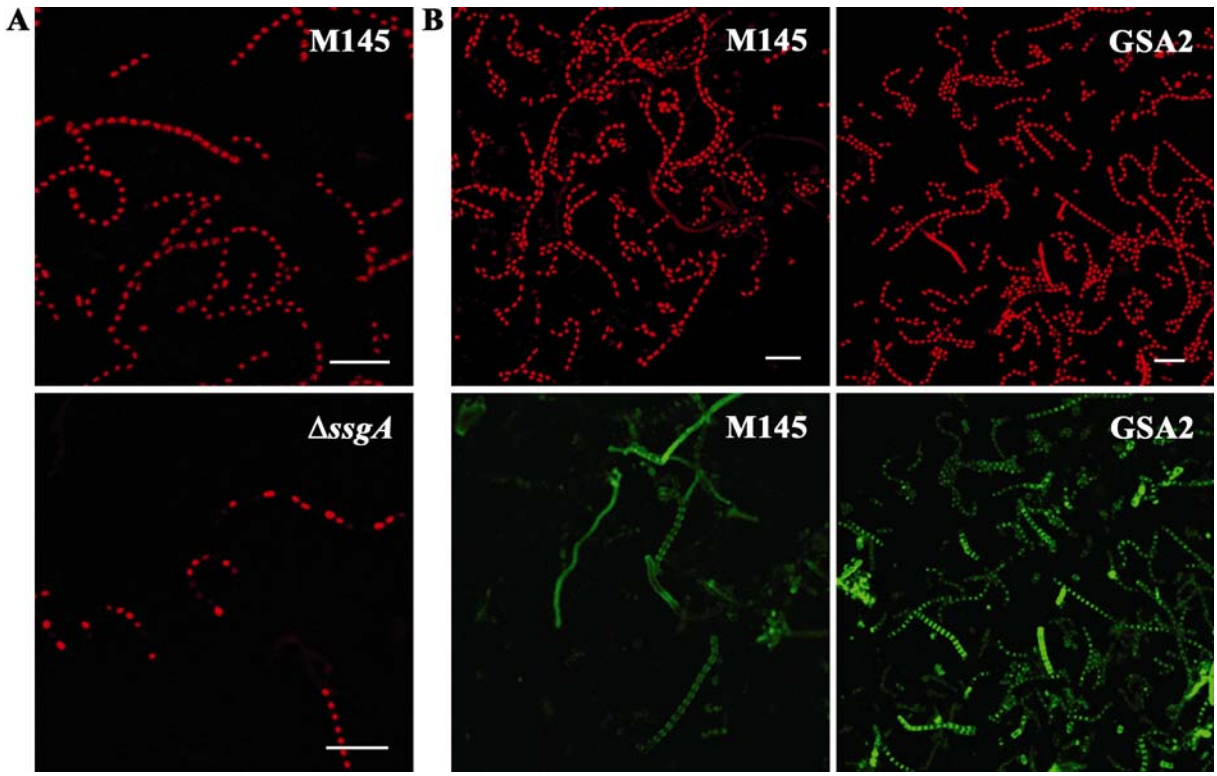


**Figure 7, Chapter 4: Visualisation of DNA and peptidoglycan subunits by fluorescence microscopy.** Cultures were grown on SFM for 5 days at 30 °C**. A.** DNA content of *S. coelicolor* and the *ssgA* mutant revealed by propidium iodide (PI). The *ssgA* mutant is disturbed in DNA segregation. **B.** *S. coelicolor* M145 and its SsgA-overexpressing derivative GSA2. Left column shows DNA visualised with PI; right column shows peptidoglycan subunits visualised with f-WGA. GSA2 shows strongly enhanced septation in young aerial hyphae. f-WGA-stained foci also were observed between spores in the mature spore chains, most likely indicative of autolysis. Bar = 5 μm.

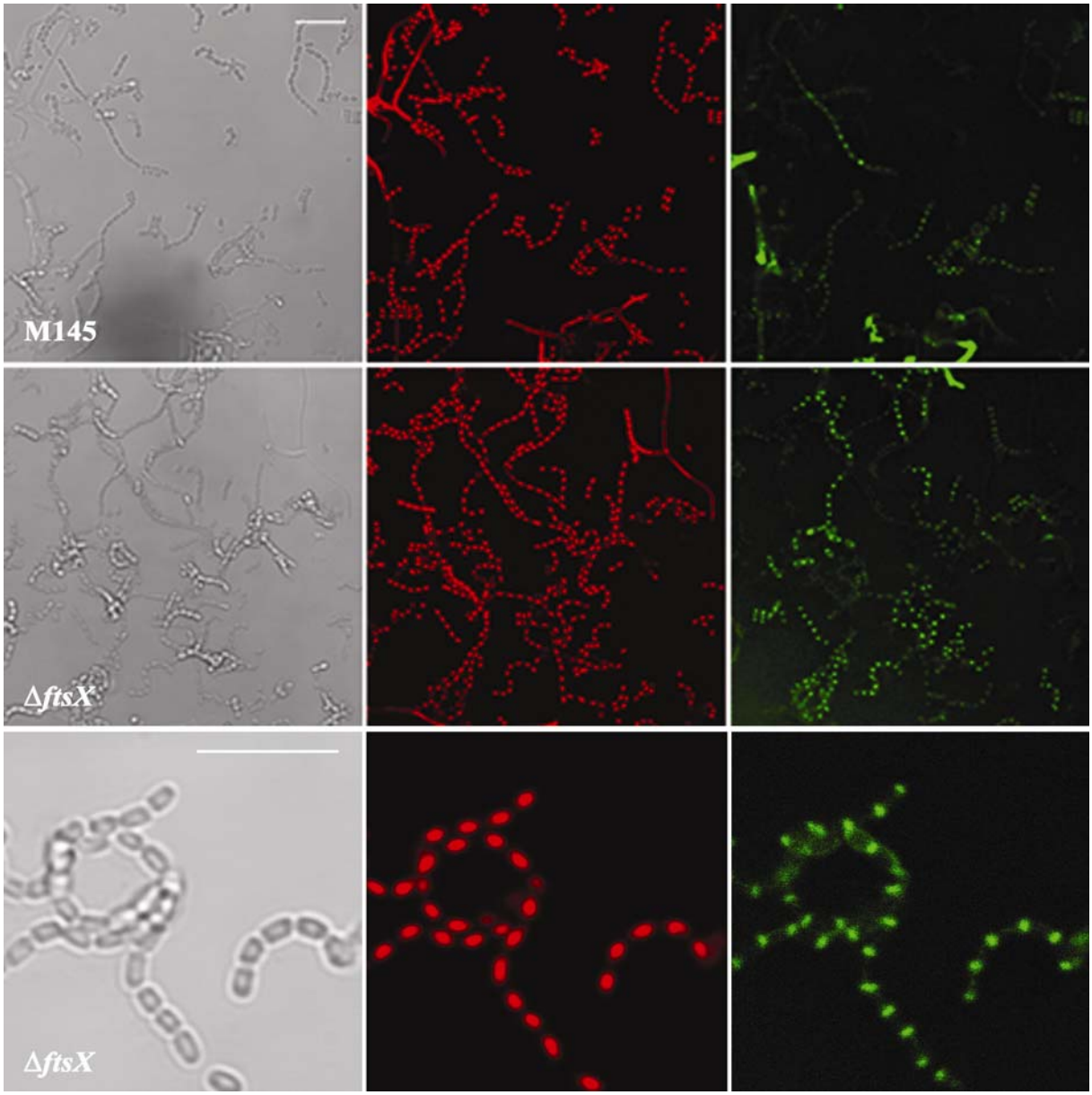


**Figure 5, Chapter 6: Analysis of an *ftsX* mutant by confocal fluorescence microscopy.** Samples were prepared from 5-day old surface-grown cultures at 30°C of the parental strain M145 and the *ftsX* mutant. DNA and peptidoglycan subunits were visualised with PI (middle column) and f-WGA (right column). The left column shows light microscopy images. Bar = 5 μm.

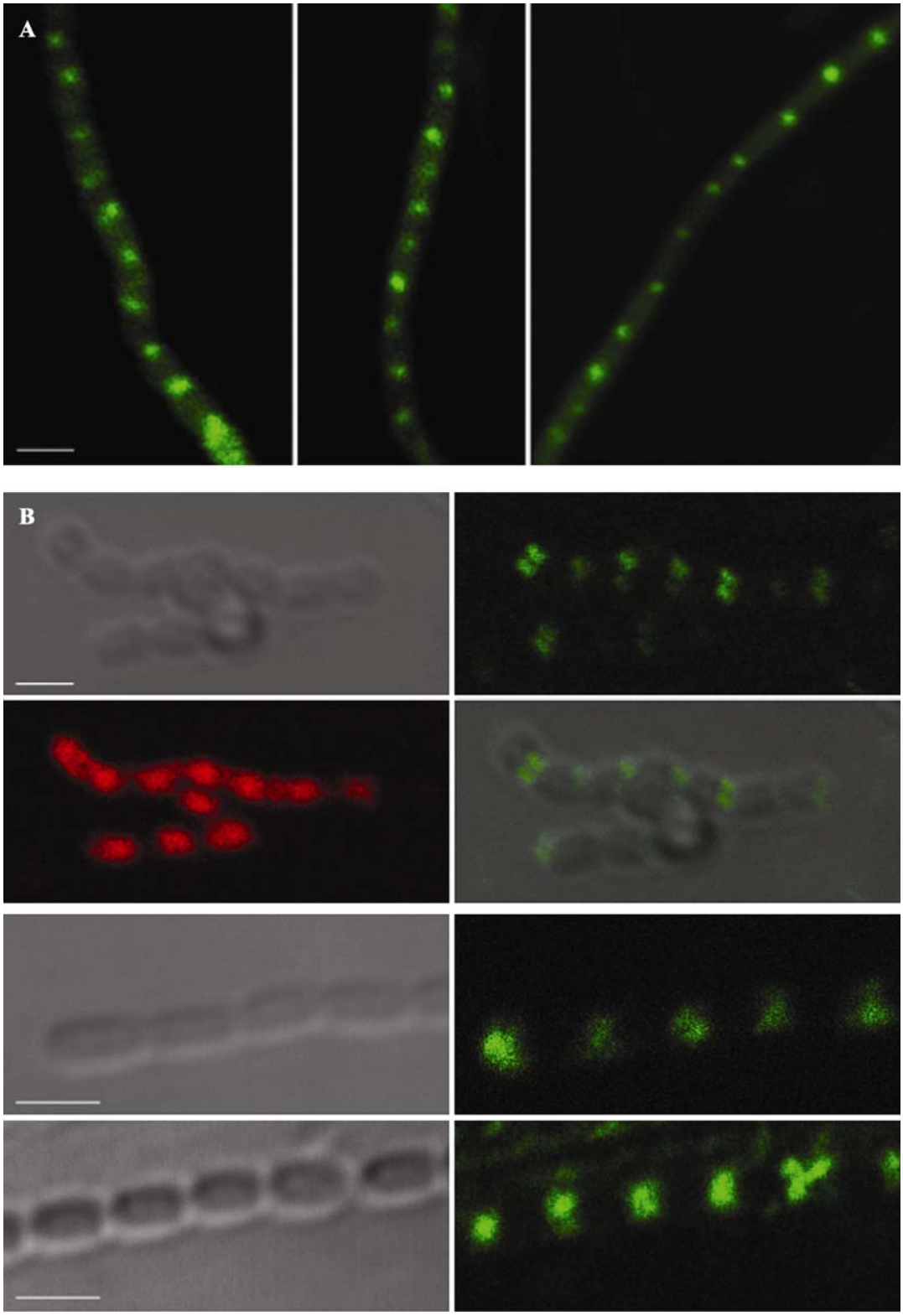


**Figure 6, Chapter 6: A. FtsZ-rings in an *ftsX* mutant.** Strains were grown on SFM for 2 days at 30°C. **B. Cellular localisation of FtsE using anti-FtsE antibodies.** Strains were grown on SFM for 5 days at 30°C. Propidium iodide was used to visualise DNA. The left column shows light microscopy images and the right column represents FtsE localisation (Row 1-3-4) or the left column shows DNA and the right column shows an overlay of the images of row 1 (Row 2). Bar = 1 μm.