

Function and control of the ssg genes in streptomyces Traag, B.A.

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Appendices

APPENDIX A. Colour figures



Chapter II. Figure 4. Dynamic localization of SsgA-GFP. Fluorescence micrographs of SsgA-GFP localization during the several stages of development of *S. coelicolor*. The width of the vegetative hyphae is around 400 nm (A, B), that of aerial hyphae and spores is around 800 nm (C-F). For further details see Noens *et al* (2007).



Chapter IV. Figure 1. Dependence of the activity of *ssg* **gene promoters on different carbon sources.** The ability to stimulate Red production by the upstream fragments of *ssgA-G* and *ssgR* (*ssgX*p) was tested on minimal medium containing mannitol (left panel) or mannitol + glucose (middle and right panel). Red production is not evidently stimulated by the promoter fragments of *ssgA*, *ssgE* and *ssgG* on minimal medium. Promoter fragments of *ssgB*, *ssgC*, *ssgD*, *ssgF* and *ssgR* stimulated Red production in M512 on mannitol (left panel), however only the promoter of *ssgD* did so on mannitol + glucose (middle panel). Promoter activity of all fragments was restored on mannitol + glucose in M512 $\Delta glkA$ (right panel).

Appendices



Chapter VI. Figure 2. Analysis by phase-contrast and fluorescence microscopy. Samples were prepared from seven days old surface-grown cultures of the parental strain M145 harboring the empty vector (**1**), the *ssgB* mutant harboring pGWS271 (*ssgB*^{SalTr}) (**2**), pGWS294 (*ssgB*^{Sery}) (**3**), pGWS298 (*ssgB*^{Acell}) (**4**) or pGWS299 (*ssgB*^{Krad}) (**5**). Note that all pictures have the same magnification. **A.** Phase-contrast light microscope pictures of spore chains. Notice the regular size of wild type spores (1) and the highly variable size of spores of the transformants (2-5). **B.** DNA was visualized by simultaneous staining with the membrane-impermeant dye Propidium Iodine (red) and the membrane-permeant dye Syto-82 (green). Light microscope images on the left correspond to fluorescent images on the right. Wild type spore chains appear in green, indicating an intact cell wall. Spore chains of the transformants contain several spores with permeated cell walls, and therefore appear in red. **Bar is 5 µm**.



APPENDIX B. The predicted structure of *S. coelicolor* SsgA

Ribbon presentation of the three-dimensional structure of *S. coelicolor* SsgA modelled to the available crystal structure of SsgB from *Thermobifida fusca* at 2.60 Å resolution (PDB 3CM1 and Ashley Deacon and Qingping Xu, pers. comm.). **A.** Front (left) and back (right) view of the predicted SsgA trimer. The three different chains are indicated in green, yellow and red. The amino acid residues L29, D58 and S89 (aa numbering according to Chapter V) that are essential for SsgA function but not conserved among other SALPs, are highlighted in black. **B.** Amino acid residues with a high *importance* score and single aa substitutions causing loss of function are highlighted in red. For details see Chapter V.