Final report Strep-Cell (ERG-230944)

Streptomycetes are soil‐dwelling filamentous bacteria that produce about 70% of all clinically used antibiotics. These bacteria grow as filaments (hyphae) that form a complex multicellular network called a mycelium. When nutrients become limiting, part of the vegetative mycelium is dismantled, concurring with antibiotic production. These secondary metabolites help to protect the pool of nutrients that is released during breakdown of the mycelium, and which is being used for a second phase of growth. During this phase, specialized hyphae are formed that grow away from the substrate into the air. This leads to the formation of spores that can survive the adverse environmental conditions. The power of actinomycetes to exploit nutrients in the soil is demonstrated by the enormous amount of secreted hydrolases, cellulases and chitinases, in total more than 100 proteins. Strikingly though, is the presence of a gene predicted to be involved in cellulose biosynthesis, rather than degradation. The putative cellulose synthase enzyme (SCO2836, hereinafter called *cslA*) is homologous to characterized cellulose synthases from other organisms, and has all the conserved catalytic residues required for cellulose synthesis. Prior to the start of the project, we discovered that this protein is important for the formation of aerial hyphae under a wide range of conditions. This suggested that CslA might have a structural role in the cell wall. However, soon after the start of this project a publication appeared describing a large part of the intended research (and partially confirming the original ideas). Therefore, the direction of the research changed, now focusing on the role of *cslA* in attachment, as well as on the identification and roles of (novel) cell surface proteins (see below).

*The role of chaplins and cellulose in attachment*

The formation of aerial hyphae is accompanied by a change in the surface hydrophobicity of hyphae. Whereas vegetative hyphae are hydrophilic, aerial hyphae and spores are hydrophobic due to the presence of a surface layer called the rodlet layer (Wildermuth et al., 1971; Claessen et al., 2002). Our group has previously discovered the proteins that form this surface layer, which are called rodlins (Claessen et al., 2002) and chaplins (Claessen et al., 2003; 2004; Elliot et al., 2003). In this Marie Curie project, we discovered that the chaplin proteins are also important for attachment of hyphae to surfaces (de Jong et al., 2009b). Attachment may be important for the effective degradation of substrates, colonization of specific niches such as the cuticle of leaf‐cutting ants and the initiation of infection processes by pathogenic streptomycetes. During hyphal attachment, an intercellular network of fimbriae is formed composed of chaplins in association with a cellulose-like polymer produced by *cslA* (Xu et al., 2008; de Jong et al., 2009b). Interestingly, Various bacteria produce cellulose during formation of biofilms and adherence to plant tissues. For instance, the extracellular matrix produced by *Salmonella enterica* comprises, in addition to curli fimbriae, cellulose and one or more other polysaccharides (White et al., 2003). Importantly, we observed detachment of the *Streptomyces* fimbriae from the cell surface by enzymatic treatment with cellulase (which degrades cellulose), revealing a critical, and previously unknown role for this polymer in fimbrial anchoring (de Jong et al., 2009b). How cellulose mediates anchoring of the fimbrial structures, and whether this is important for fimbrial anchoring in pathogenic microbes, is still under current investigation. Results from such studies might be important to get a better understanding of the mechanisms that enable pathogenic organisms to interact with their host, and might lead in the long term to the development of an entire new class of drugs.

*The role of NepA in control of spore germination*

Previous work from our group strongly suggested the existence of a regulatory mechanism, which is activated as soon as aerial growth commences. This then leads to the expression of many aerial‐hyphae specific genes (Claessen et al., 2004; 2006). We have compared whole genome expression of a wild type colony of *Streptomyces coelicolor* forming aerial hyphae and spores with that of the *chp* null mutant that forms few aerial structures. This revealed that expression of 244 genes was significantly altered, among which genes known to be involved in development. One of the genes that was no longer expressed in the ∆*chpABCDEFGH* mutant was *nepA*, which was previously shown to be expressed in a compartment connecting the substrate mycelium with the sporulating parts of the aerial mycelium (Dalton et al., 2007). However, expression is also detected in developing spore chains, where NepA is secreted to end up as a highly insoluble protein in the cell wall (de Jong et al., 2009a). Germination of spores of a *nepA* deletion mutant was faster and more synchronous, resulting in colonies with an accelerated morphogenetic programme. Crucially, spores of the *nepA* mutant also germinated in water, unlike those of the wild‐type strain. Taken together, NepA is the first bacterial structural cell wall protein that is important for maintenance of spore dormancy under unfavorable environmental conditions (de Jong et al., 2009a).

*Publications and other impact*

The research carried out during this project has been described in four publications, including two in one of the leading microbiology journals *Molecular Microbiology*. Moreover, the work has been presented at various international research meetings. The reintegration grant has also contributed to the establishment of an independent research position for the fellow at Leiden University, where part of the research will be continued.

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