CELL WALL POLYSACCHARIDE INVOLVED IN PELLET FORMATION IN STREPTOMYCES LIVIDANS 1326

Giulia Mangiameli, Gilles Van Wezel, Erik Vijgenboom; Molecular Biotechnology, IBL, Leiden University, P.O. Box 9505, 2300 RA Leiden, g.mangiameli@chem.leidenuniv.nl

Streptomyces, pellet, polysaccharides

Introduction. Streptomycetes are Gram positive soil bacteria that grow as a mycelium and display a complex morphological and physiological development. *Streptomyces lividans* has shown potential as a host for the production of proteins of pharmaceutical and industrial relevance. However, its commercial application has been hampered so far due to the limitations dictated by its mycelial growth. Changing the level of fragmentation by morphological engineering through the overexpression of SsgA does increase enzyme production[1]. A screen of new morpho gene targets in *S. lividans* came up with two candidates of interest, encoding a glycosyl transferase (SLI3187) and a radical copper oxidase (SLI3188). Both are cell wall associated and take part in the synthesis and/or modification of polysaccharides in the cell wall. The involvement of polysaccharides in biofilm and pellet formation is a topic in various species including *Streptomyces*[2]. The deletion of the SLI3187 ortholog in *S. coelicolor* (SCO2836) results in smaller pellets in liquid cultures[3,4]. However, the actual enzyme activity of 3188 and 3187, their substrate and product have not been established.
In this work we aim to elucidate the function of 3188 and 3187 in extracellular matrix formation and pellet size in *S. lividans* 1326 and to identify the polysaccharide produced.

Methods. Single and double deletion mutants were made in *S. lividans* with the Cre-lox technology[5]. Localization of GFP-fused proteins and polysaccharide stain were visualized by fluorescent microscopy. Classical protocols for *Streptomyces* cloning were applied [6]

Results. The Δ*3187* and Δ*3188* mutants both showed the same open mycelium/pellet structure when grown in liquid rich media. Concurrently the mutants lost the ability to attach to the surface of a number of solid (agar) substrates. Moreover, the two proteins show the same localization in actively growing hyphal tips. Specific dyes revealed the absence of polysaccharides with β-1,4 linkages in the hyphal tips of both mutants, underlying the involvement of the proteins in the same biological process. Identification of the polysaccharide is in progress using GFP labeled CBDs.

Conclusions. In this work we demonstrate the role of 3187 and its functional partner 3188 in the process of synthesis and/or modification of a cell wall associated polysaccharide in *S. lividans*. Deleting one or both of the genes encoding these proteins has a significant effect on pellet size and the ability of the mycelium to attach to solid surfaces. The impact of the mutant phenotype on the level of enzyme expression is currently under investigation.

Acknowledgements. This work was supported by an ERA-IB network grant (EIB.08.013).

References.

1. Van Wezel GP, Krabben P, Traag B, Keijser BJ, Kerste R, Vijgenboom E, Heijnen JJ, Kraal B. (2006). Unlocking Streptomyces Spp. for Use as Sustainable Industrial Production Platforms by Morphological Engineering. *Applied and Environmental Microbiology*. 72,(8.):5283–8.
2. Kim YM, Kim J.(2004). Formation and Dispersion of Mycelial Pellets of Streptomyces coelicolor A3(2). *Journal of Microbiology (Seoul, Korea)*. 42,(1.): 64–7.
3. Xu H, Chater KF, Deng Z, Tao M. (2008). A cellulose synthase-like protein involved in hyphal tip growth and morphological differentiation in streptomyces. *Journal of bacteriology*. 190,(14.): 4971–8.
4. Van Veluw GJ, Petrus ML, Gubbens J, de Graaf R, de Jong IP, van Wezel GP, Wösten HA, Claessen D. (2012). Analysis of Two Distinct Mycelial Populations in Liquid-grown Streptomyces Cultures Using a Flow Cytometry-based Proteomics Approach. *Applied Microbiology and Biotechnology*. 96,(5.): 1301-12
5. Fedoryshyn M, Welle E, Bechthold A, Luzhetskyy A (2008). Functional Expression of the Cre Recombinase in Actinomycetes. *Applied Microbiology and Biotechnology*. 78, (6.): 1065–70.
6. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. (2000). *Practical Streptomyces Genetics*. John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, England.