The cellobiose-sensor CebR is the gatekeeper of *Streptomyces scabies* pathogenicity

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Introduction

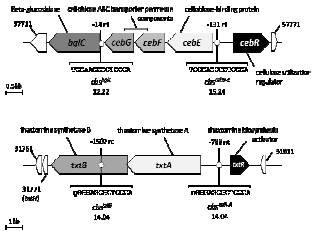
Streptomyces is a very large genus of Gram-positive, high G+C content bacteria that are mostly saprophytes and best known for the production of pharmaceutically- and agriculturally-important secondary metabolites. Although several hundred species are known to date, only a few are phytopathogenic. One of the best studied pathogen is *Streptomyces scabies* which cause raised or pitted scab lesions on economically-important root and tuber crops like potato, radish, beet, peanut, and sweet potato. The pathogenicity of *S. scabies* is strictly associated with the levels of production of the phytotoxin thaxtomin A which functions as a potent and non- discriminative plant cellulose synthesis inhibitor. It is known that this phytotoxin production is induced by multiple signals that originate from plant material such as xylan-degradation products, suberin, and, more importantly, cellobiose, a product of cellulose degradation and a well-known elicitor of thaxtomin biosynthesis in *Streptomyces scabies*.

Potato common scab caused by *S. scabies*

This work aims at elucidating the molecular mechanism by which the phytopathogenic species *Streptomyces scabies* coordinates sensing of cellobiose originating from plant cell wall degradation and the onset of pathogenicity *via* the production of its main virulence factor, the phytotoxin thaxtomin A. To achieve this goal, we used the PREDetector software in order to identify some putative *cis*-acting elements of CebR, the cellulose utilization regulator, within genome of *S. scabies.*

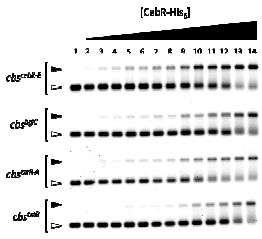
Results

Predicted CebR-binding sites involved in thaxtomin biosynthesis



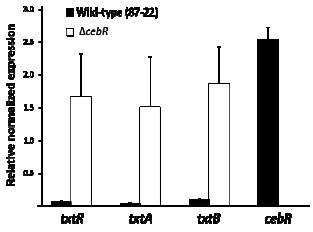
Predicted CebR-binding sites found with the PREDetector software in *S. scabies* genome

In vitro validation of the predicted CebR-binding sites



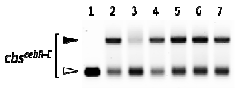
Electromobility shift assay (EMSA) carried out with fluorescent probes correpsonding to each predicted CebR-binding sites and increasing concentrations of purified 6His-CebR.

Effect of *cebR* deletion in *S. scabies* on the transcription levels of the thaxtomin biosynthetic and regulatory genes



Quantitative real-time RT-PCR analysis of gene expression levels in *S. scabies* 87-22 and in the Δ*cebR* strain.

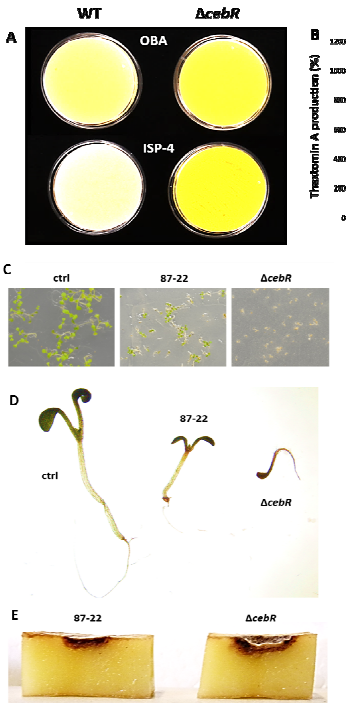
Effect of cellooligosaccharides on CebR DNA-binding ability



Electromobility shift assay (EMSA) performed with a fixed concentration of 6His-CebR and different cellooligosaccharides. 1: probe alone 2: no sugar 3: cellobiose 4: cellotriose 5: cellotetraose 6: cellopentose 7: cellohexose.

Results

Deletion of *cebR* results in constitutive thaxtomin A production and hypervirulence of *S. scabies*

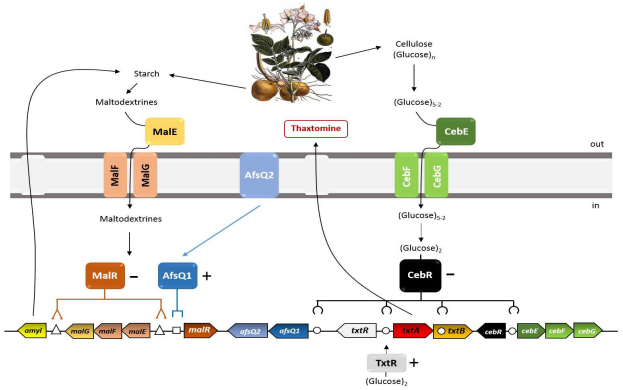


A. Deletion of *cebR* in *S. scabies* resulted in higher thaxtomin production (yellow pigmentation) on OBA and even in production under conditions which do not trigger thaxtomin production in the wild type strain 87-22. **B.** HPLC analysis of thaxtomin A extracted from plates shown in A. **C**. Phenotype of *Arabidopsis thaliana* grown eight days in the presence of *S. scabies* 87-22 (wild-type) and its *cebR* null mutant. **D.** Close up of representative plants grown in MS plates shown in C. **E.** Potato tuber slice assay.

Conclusions and perspectives

In this work, we highlighted the major expression locking system of thaxtomin biosynthesis in *Streptomyces scabies* where CebR, the cellulose utilization regulator, acts as the master repressor of the path to pathogenicity.

The elucidation of the molecular mechanisms associated with thaxtomin production and thereby in triggering the pathogenic behavior of *Streptomyces scabies* was essentially investigated through the screening of signaling molecules that induce the biosynthesis of this phytotoxin. In this study we undertook an *in silico* approach which aimed to identify a series of *cis*-acting elements of well-characterized DNA-binding proteins possibly involved in the control of thaxtomin production. This approach allowed us to demonstrate that the cellobiose-induced thaxtomin production in *Streptomyces scabies* is due to the release of CebR from its DNA-binding sites associated with the *txt* biosynthetic/regulatory cluster. Interestingly, next to this major regulatory mechanism, our bioinformatic analysis suggests the occurrence of a complex regulatory network from cellobiose sensing to starch utilization.



Hypothetical pathway built using predicted CebR/AfsQ1-binding sites found with the PREDetector software in *S. scabies* genome

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