Name(s)
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Project Number
38087

Project Title
The Effect of Mutating Cellobiose Transporters on Thaxtomin Production in the Plant Pathogen Streptomyces scabies

Abstract

Objectives/Goals
Pathogenicity by the plant pathogenic Streptomyces scabies, the causative agent of common scab disease on various economically important root and tuber crops, is triggered by cellobiose, a subunit of the plant cell wall polymer cellulose. Cellobiose induces the production of thaxtomin A, the main virulence factor of this species. This phytotoxin affects the plant cell wall leading to stunted growth, cell hypertrophy and tissue necrosis. Previous research found that the deletion of the primary cellobiose transporter resulted in a significant decrease in thaxtomin production. However, bacteria missing this transporter were still viable on minimal medium with cellobiose as the only carbon source (TDMc). Hence, the presence of another cellobiose transporter was suspected. Indeed, homology searches revealed there to be two other transporter candidates.
The goal of this project is to study the role of these additional transporters during the onset of plant pathogenicity of S. scabies.

Methods/Materials
Deletion mutants were created by replacing the gene coding for the solute-binding protein of the transporters by an antibiotic resistance cassette. Three assays were conducted. For each assay, cultures of bacteria were grown and the optical density was equalized. Three biological repeats were present in all assays. The liquid and plate assays had two technical repeats. These assays were conducted on thaxtomin dependent medium with cellobiose (TDMc) and oat bran medium liquid (OBB) and solid (OBA), both of which are complex mediums. The radish assay was conducted on 1.5% agar. Thaxtomin production was measured through an HPLC machine.

Results
The mutation of second and third transporters showed no difference in growth or thaxtomin production compared to the wild type when grown on TDMc. However, on OBA one of the mutants failed to produce toxin. In addition, radish seedlings infected with this mutant only showed an attenuated virulence phenotype.

Conclusions/Discussion
The results show that under complex conditions at least one other transporter is important in the sensing of environmental triggers inducing the production of thaxtomin. The loss of a second transporter could not be compensated for by the actual cellobiose transporter. This is shown by the results of plant bioassays and the inability of this mutant to produce toxin on plant-based media that are known to induce thaxtomin production.

Summary Statement
At least two transporters are involved in the sensing of environmental triggers that induce the production of the plant toxin thaxtomin A in the plant pathogenic bacterium Streptomyces scabies.

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