DTU Bioengineering
Department of Biotechnology and Biomedicine

Technical University of Denmark

Studying *B. subtilis* root colonization of different plant species

Master student Christopher Blake

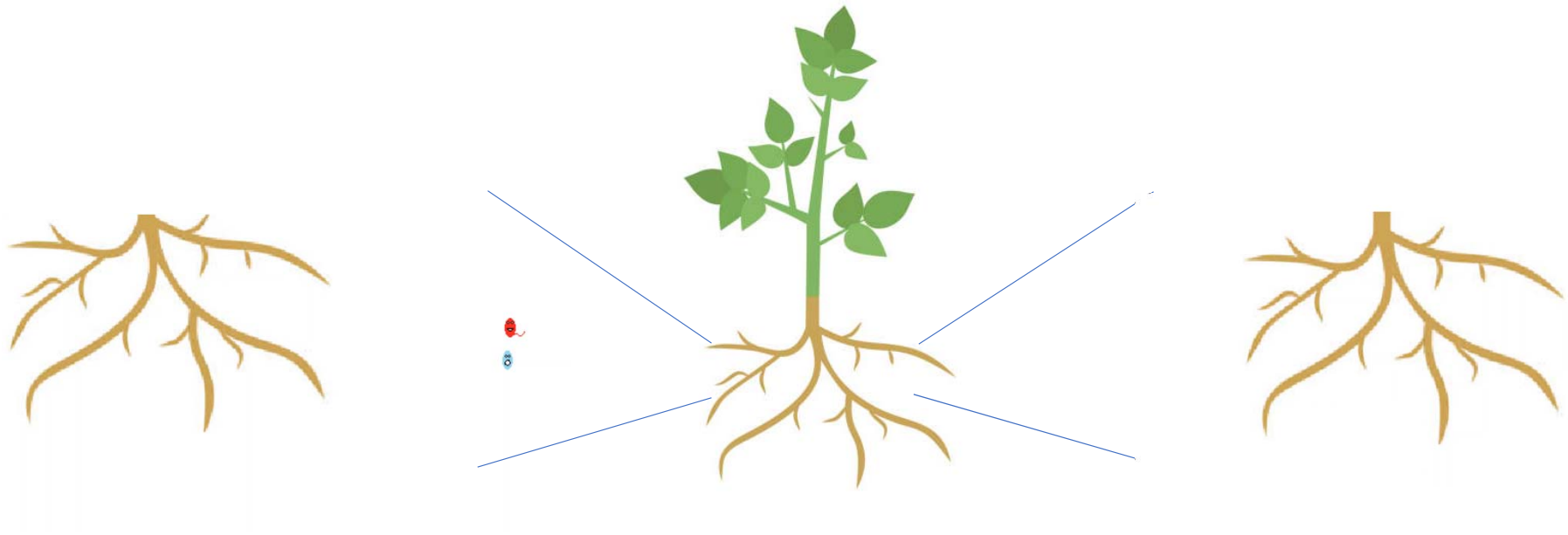
Supervisor: Prof. Ákos T. Kovács
Co-supervisor: PhD student Mathilde Nordgaard Christensen

14th November 2019

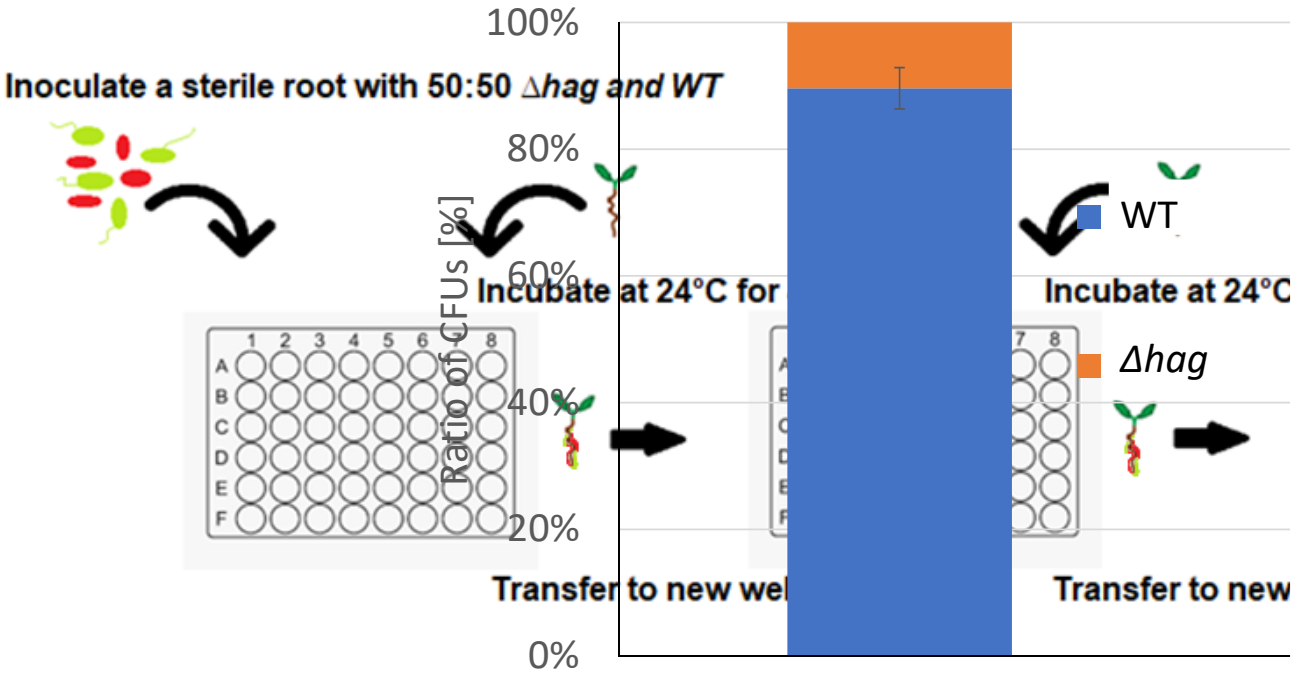
Important properties for root colonization by *B. subtilis*

Motility

Biofilm formation



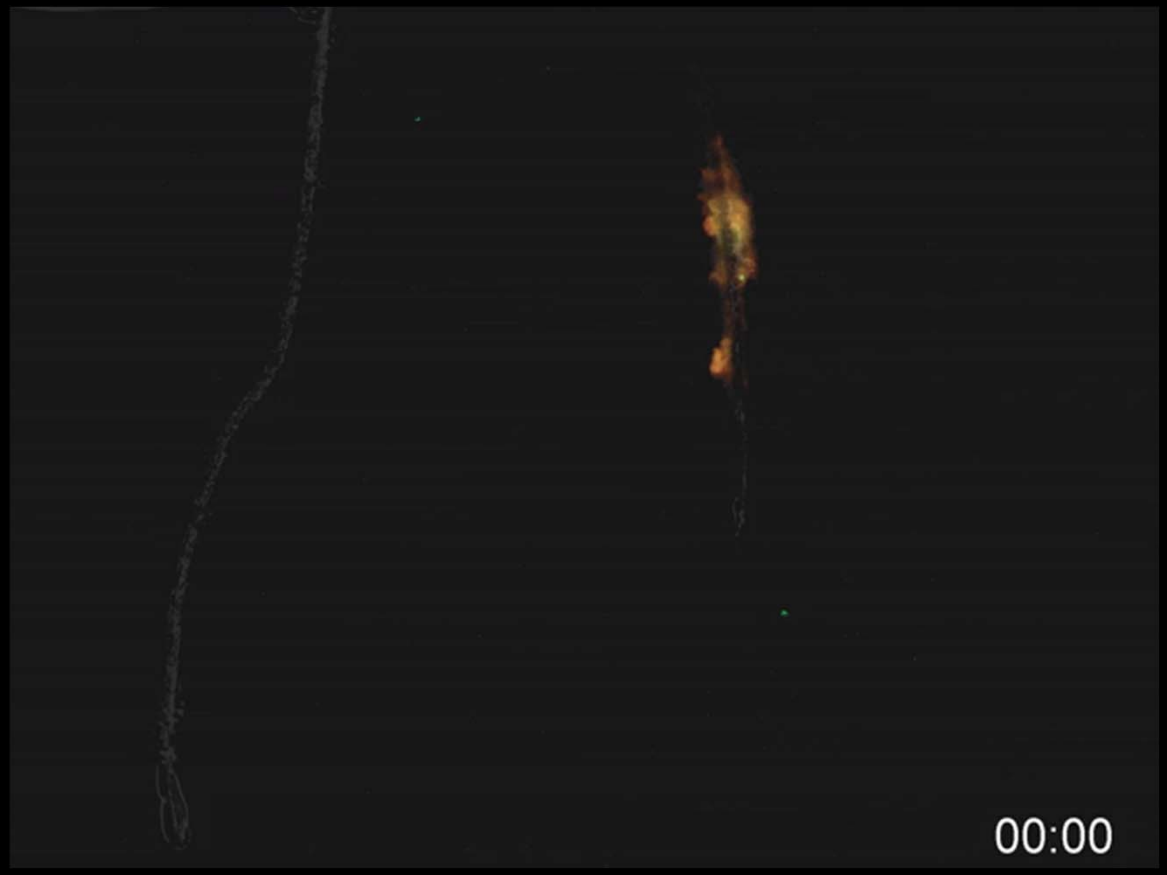
A. thaliana root re-colonization of flagellin deficient *Δhag* mutant in competition with WT



Δhag vs. WT (non-shaken)

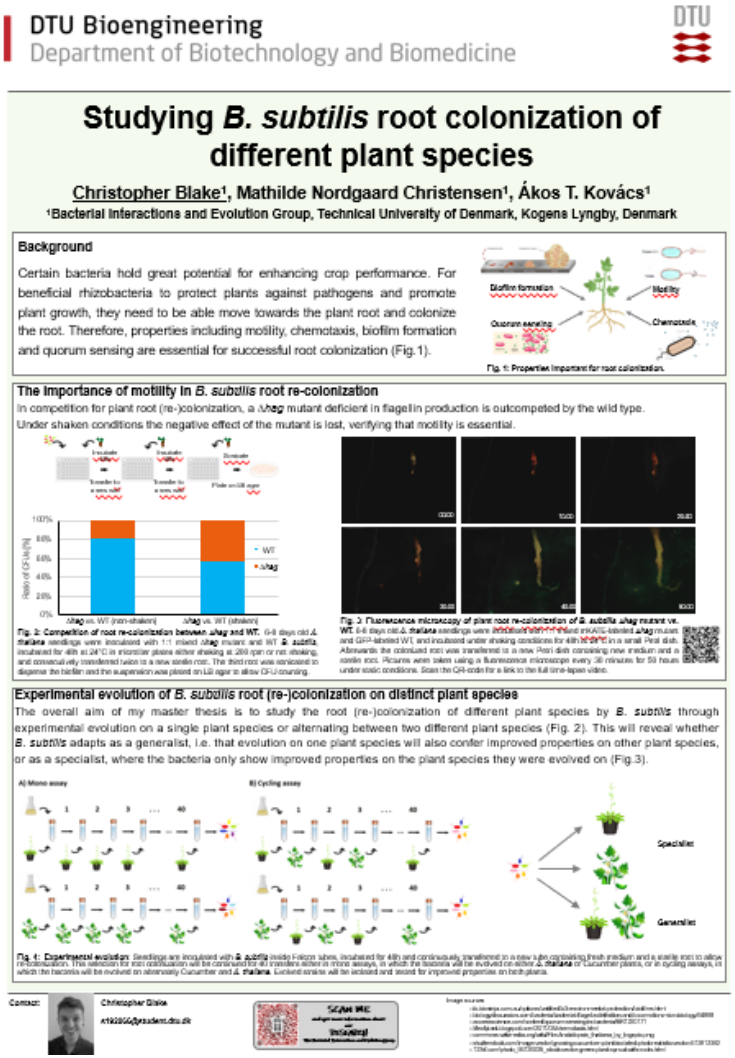
Δhag vs. WT (shaken)

Δhag = red
WT = green



Thank you for your attention!

My poster is in the final poster session (11:05-11:50)



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Studying *B. subtilis* root colonization of different plant species

Christopher Blake[1], Mathilde Nordgaard Christensen[1], Ákos T. Kovács[1]
[1]Bacterial Interactions and Evolution Group, Technical University of Denmark, Kogens Lyngby, Denmark

Background

Certain bacteria hold great potential for enhancing crop performance. For beneficial rhizobacteria to protect plants against pathogens and promote plant growth, they need to be able move towards the plant root and colonize the root. Therefore, properties including motility, chemotaxis, biofilm formation and quorum sensing are essential for successful root colonization (Fig.1).

Fig. 1: Properties important for root colonization.

The importance of motility in *B. subtilis* root re-colonization

In competition for plant root (re-)colonization, a Δ*hag* mutant deficient in flagellin production is outcompeted by the wild type. Under shaken conditions the negative effect of the mutant is lost, verifying that motility is essential.

Fig. 2: Competition of root re-colonization between Δ*hag* and WT. 6-8 days old *A. thaliana* seedlings were inoculated with 1:1 mixed Δ*hag* mutant and WT *B. subtilis*, incubated for 48h at 24°C in microtiter plates either shaking at 200 rpm or not shaking, and consecutively transferred twice to a new sterile root. The third root was sonicated to disperse the biofilm and the suspension was plated on LB agar to allow CFU counting.

Fig. 3: Fluorescence microscopy of plant root re-colonization of *B. subtilis* Δ*hag* mutant vs. WT. 6-8 days old *A. thaliana* seedlings were inoculated with mKATE-labeled Δ*hag* mutant and GFP-labeled WT, and incubated under shaking conditions for 48h at 24°C in a small Petri dish. Afterwards the colonized root was transferred to a new Petri dish containing new medium and a sterile root. Pictures were taken using a fluorescence microscope every 30 minutes for 50 hours under static conditions. Scan the QR-code for a link to the full time-lapse video.

Experimental evolution of *B. subtilis* root (re-)colonization on distinct plant species

The overall aim of my master thesis is to study the root (re-)colonization of different plant species by *B. subtilis* through experimental evolution on a single plant species or alternating between two different plant species (Fig. 2). This will reveal whether *B. subtilis* adapts as a generalist, i.e. that evolution on one plant species will also confer improved properties on other plant species, or as a specialist, where the bacteria only show improved properties on the plant species they were evolved on (Fig.3).

Fig. 4: Experimental evolution. Seedlings are inoculated with *B. subtilis* inside Falcon tubes, incubated for 48h and continuously transferred to a new tube containing fresh medium and a sterile root to allow re-colonization. The selection for root colonization will be conducted for 40 transfers either in mono assays, in which the bacteria will be evolved on either *A. thaliana* or cucumber plants, or in cycling assays, in which the bacteria will be evolved on alternately cucumber and *A. thaliana*. Evolved strains will be isolated and assed for improved properties on host plants.

Contact:
Christopher Blake
s192666@student.dtu.dk

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