Establishment of a system for bacterial colonization experiments in wheat

Dissertation

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Submitted by

Ryszard Soluch

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First referee: Prof. Dr. Tal Dagan

Second referee: Prof. Dr. Eva H. Stukenbrock

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1 Abstract

Eukaryotes, including plants, are hosts to microbes. These microbes can be recruited from the environment as well as transferred between the generations horizontally or vertically. We hypothesize that in wheat, due to the priority effects, vertically transferred microbiota has priority advantage over bacteria recruited from the environment.

Isolation efforts on *Triticum aestivum* seeds and seedlings have yielded few cultivable bacterial strains present both on and in the seed and seedling. *Pantoea agglomerans* was the species found in most of the samples, independent of the geographical origin (Germany, Turkey).

Here we show that the GFP-tagged strain of *Pantoea agglomerans*, originally isolated from wheat, maintains its ability to colonize germ-free wheat roots and to proliferate within the root. Independently of the inoculum size used, log-phase-like population growth within the root was observed. Afterward, while the population growth was still ongoing, any further increase in the population size was tied to the growth of the root habitat. Once the population count was standardized based on root fresh weight, the plateau was observed within 8 days post-inoculation, showing that the carrying capacity of the host is limited.

In planta competitions between GFP and mCherry tagged *Pantoea* strains were performed to check for numerical priority effects and adaptive priority effects. Competing strains were introduced with a time lag between them, or one of the strains was pre-cultivated in a plant, while the second was cultivated in liquid media.

Our results show a strong impact of the priority effect. The numerical advantage of primary colonizer led to its high relative abundance. No adaptive advantage was observed. Compared to our single colonizer experiments, total carrying capacity of the host was unchanged.

Persistence of *Pantoea* in the plant host was tested up to 46 days post-inoculation. Density of the population varies between the plant compartment, reaching highest CFU per gram of tissue in the roots, lowest in the leaves.

My observations support the hypothesis of vertical transfer of *Pantoea agglomerans* and its association with host lifecycle (ability to colonize the seedling from the seed while outcompeting soil-borne *Pantoea*).

This data provides the fundament for future experiments on mechanistic aspects of wheat colonization by *Pantoea agglomerans*, which can be performed by analysis of the bacterial genome and preparation of knock-outs. The established gnotobiotic system enables further competition and exclusion experiments.