HARMLESS OR DEADLY?

Adaptation of Insecticidal Pseudomonads to Insects

BASIC INFORMATION

Thesis type: Semester project or master thesis
Advisor/direct supervisor: Maria Zwyssig
Formal supervisor: Monika Maurhofer
Start date: open

BACKGROUND

Plant beneficial pseudomonads are promising candidates for pest control in agriculture[1]. The subgroups *Pseudomonas protegens* and *P. chlororaphis* are efficient root-colonizers and harbor besides disease-suppressive[2,3,4] also insecticidal activities against plant insect pests[5,6,7,8]. Although many traits enabling root colonization and insect pathogenicity are already known, it is not clearly understood how these bacteria have adapted to living in an insect[9] and how they switch between their lifestyles[8]. Furthermore, it is unsure if their relationship towards insects is pathogenic in general or if it is sometimes commensal towards certain insect species[9]. We are performing an experimental evolution with *P. protegens* CHA0 based on repeated feeding cycles to larvae of the crop pest *Plutella xylostella*. Analyzing the evolved clones for genomic changes and alterations in insect- and plant-bacteria interactions will provide new insights on the adaption of plant beneficial pseudomonads to insects as well as how they switch between their lifestyles.

OBJECTIVES

The goal is to compare the phenotype and the genotype of the evolved clones to the original strain. Phenotypes that will be characterized are oral insecticidal activity and the root- and insect-colonization ability. The definitive objectives and therefore also the tasks and methods will depend on the progress of the experiments at the start date and can be adapted to the interests of the student.

TASKS AND METHODS

Oral insecticidal activity against insect pests

- Plutella feeding assays will be repeated at the end of the evolution cycles to compare the oral insecticidal activity and the insect-colonization ability of the evolved to the non-evolved clones of *P. protegens* CHA0.
- *Plutella xylostella* larvae will be fed with lower, non-lethal inoculum concentrations to compare the persistence of the evolved clones over different life stages of this insect.
- Radish pot assays will be performed to screen for increased biocontrol efficiency of the evolved clones.

Root colonization and plant protection

- The ability of the evolved clones to persist in the rhizosphere and colonize plant roots will be analyzed by CFU determination of soil and radish roots over time.
- Plant protection ability of the evolved clones against plant diseases like *Pythium ultimum* will be tested in a greenhouse setup.

Comparative genomics

- The genomes of interesting evolved clones will be compared using comparative genomics approaches.
- Interesting genes will be knocked-out in the unevolved clone and the phenotype will be analysed.

REFERENCES

1. Kupferschmied, P., Maurhofer, M. & Keel, C. Promise for plant pest control: root-associated pseudomonads with insecticidal activities. *Front. Plant Sci.* **4**, 287 (2013).
2. Pieterse, C. M. J. *et al.* Induced Systemic Resistance by Beneficial Microbes. *Annu. Rev. Phytopathol.* **52**, 347–75 (2014).
3. Haas, D. & Défago, G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* **3**, 307–319 (2005).
4. Lugtenberg, B. & Kamilova, F. Plant-Growth-Promoting Rhizobacteria. *Annu. Rev. Microbiol.* **63**, 541–556 (2009).
5. Péchy-Tarr, M. *et al.* Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens*. *Environ. Microbiol.* **10**, 2368–2386 (2008).
6. Ruffner, B. *et al.* Ruffner, B. et al. Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens *Photorhabdus* and *Xenorhabdus*. *BMC Genomics* **16**, 609 (2015).
7. Flury, P. *et al.* Insect pathogenicity in plant-beneficial pseudomonads: Phylogenetic distribution and comparative genomics. *ISME J.* **10**, 2527–2542 (2016).
8. Vesga, P. *et al.* Transcriptome plasticity underlying plant root colonization and insect invasion by Pseudomonas protegens. *ISME J.* **14**, 2766–2782 (2020).
9. Vesga, P. *et al.* Unpublished data