Tn*5* mutagenesis as a method for determination of necessary genetic features of bacterial antagonists

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Fire Blight is a bacterial disease which attacks plants of the Rosaceae family. It can lead to great economic losses, especially in the area of fruit-production. An effective control strategy is based on streptomycin, but application of antibiotics in plant production is highly discussed. An approach on biological level depends on control by application of bacterial antagonists. These organisms are able to fight the Fire Blight pathogen *Erwinia amylovora* for example by competition or by production of toxic compounds.

Possible antagonists against *E. amylovora* are the closely related epiphyte *Erwinia tasmaniensis* and representatives of the genus *Bacillus*. *Bacilli* are often characterized by their ability to produce a broad spectrum of secondary metabolites with toxic effects on Gram-negative bacteria. Nevertheless, there might be other factors responsible for such inhibitory abilities. Identification of features involved in the pathogen-antagonist interaction could be a useful criterion for antagonist selection.

A screening method for antagonists *in planta* has been introduced for inhibition of *Pseudomonas syringae* DC3000 by *Sphingomonas* sp. Strain Fr1 in a model system of *Arabidopsis thaliana*. This setup seems promising and might be adapted to the Fire Blight pathosystem as well, using pear slices, apple flowers or *in vitro* plantlets for screening.

Tn*5* transposon mutagenesis allows generation of random mutations on chromosomal level. The plasmid pRL27 carries a hyperactive transposase which leads to high frequency transposon transfer. Another advantage of this transposon is a conditional origin of replication which allows an easier way of re-isolation by rescue cloning for further investigations. Using a luminescent *E. amylovora* strain as an indicator enables screening for antagonist mutants which have lost their inhibitory effect on pathogen growth. These measurements are feasible in micro titer plates which allow a high-throughput screening.

Determination of genetic features which are important for antagonistic abilities enables comparison to other pathosystems and might be useful to identify suitable antagonists in different areas. It also could be used for preselection of antagonistic organisms before application in field trials which have only a limited capacity for testing.