Investigation of molecular mechanisms involved in *Erwinia amylovora* biofilm formation

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Key message. In this study we generated four insertional *Erwinia amylovora* mutants within *bssS* gene and *waa*, *aaeXAB* and *hec* operons. Our research included studying of the phenotypic characteristics of these mutants.

Keywords: biofilm formation, *Erwinia amylovora*, fire blight

A devastating plant disease fire blight, caused by the Gram-negative bacterium *Erwinia amylovora*, represents an enormous threat to fruit cultivation, as it can infect most plants of the *Rosaceae* family like apple, pear, rowanberry, quince. A variety of *E. amylovora* virulence factors have been described, and the majority of these, such as the type III secretion system (T3SS), the exopolysaccharides amylovoran and levan were found to contribute to the establishment of a successful infection in Rosaceous plants [1]. Biofilm formation may play an important role in the pathogenesis of *Erwinia amylovora* and in the movement of bacterial cells via plant xylem.

Bioinformatic analysis of the complete genome sequence of *E. amylovora* E2 have revealed several genes potentially involved in biofilm formation. In order to study the function of *rfa* (*waa*) operon (lipopolysaccharide (LPS) biosynthetic gene cluster), *aaeXAB* operon (aromatic carboxylic acid efflux operon), *hecAB* operon (involved in cell attachment and aggregation to the surfaces) and *bssS* (*yceP*) gene (regulator of biofilm through signal secretion) in the biofilm formation of *E. amylovora*, we generated four insertional mutants within these loci. Mutants were constructed by using the PCR-based one-step inactivation technique, as described previously [2]. Primer pair flanking the target loci and internal primers was used to confirm the mutants by PCR. Further research will be focused on studying the phenotypic characteristics of mutation in selected genes (*in vitro* biofilm assay, determining amylovoran concentration, cellulose biosynthesis assay) and identification of major mechanisms for regulating gene expression in *E. amylovora* biofilms.

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