

FIRE-BLIGHT PATHOGEN *ERWINIA AMYLOVORA*, PERSISTING IN UKRAINE: MULTI-APPROACH ANALYSIS

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The enterobacterium *Erwinia amylovora* (*Eam*) is a devastating plant pathogen causing necrotrophic fire blight disease of rosaceous plants that has global economic importance for apple and pear production and trade. The urgency of finding an efficient method of accurate identification is due to importance to control its prevalence. Understanding of pathogen's biology, as well as studying its closely-related species, can promote the development of antibacterial products and preventive measures, including those based on bacteriophages.

With a 5-year time interval several strains of *Eam* were isolated in Western part of Ukraine from affected quince, pear, apple trees, rowan-tree and pyracantha. Their biochemical, serological, pathogenicity properties and phage susceptibility were studied. Here we add the analysis with such features as the ability of mobile genetic elements maintaining, as well as fatty acid (FA) content, being compared to those of *Erwinia "horticola"* (*Eho*) – a closely-related pathogen causing similar symptoms in beech and apple trees, isolated earlier in Ukraine. Reference collection strains *Eam* 9057 and K8 were also included in the analyses.

Both the newly isolated and collection *Eam* strains carry a plasmid replicon of the same size. We presume it to be a pEA29, the most common extrachromosomal DNA in the abovementioned species. Notably, in most strains (except for typical strain *Eam* K8) it can be extracted using only harsh conditions. On the contrary, in *Eho* the variability of replicons is much bigger: from absence of any extrachromosomal DNA up to 2 and 3 large replicons. In *Eho*, as well as in strain *Eam* K8 plasmids can be easily extracted under standard conditions.

According to their FA content, the isolated *Eam* strains appeared to be a homogeneous group. Both major FA (represented by C12:0, C14:0, C16:0; C16:1, C18:1, and C17:0cyc) and minor FA percentage fully coincided with that of collection strain *Eam* 9057. Comparing to *Eho* strains, in *Eam* saturated even FA content was higher: $48.43 \pm 0.92\%$ vs $39.30 \pm 1.36\%$ in *Eam* and *Eho* respectively, while saturated odd FA portion was rather underrepresented: $1.86 \pm 0.52\%$ vs $5.25 \pm 1.67\%$. The content of unsaturated FA and hydroxy-FA was more or less similar. The major difference was revealed in in cyclic FA, being as low $2.22 \pm 0.38\%$ in *Eam* strains, while $9.19 \pm 2.40\%$ in *Eho*.

Again, this was not relevant for *Eam* K8: on a tree, constructed on a basis of FA content distances between strains of *Eam* and *Eho*, this strain did not cluster within *Eam* group, while appeared in a cluster of *Eho* species. Also, *Eho* as a species was revealed to represent rather heterogeneous group, varying in FA content more severely than *Eam*.

Notably, newly isolated *Eam* strains and collection strain *Eam* 9057 remain absolutely resistant to phages isolated from the same material as the bacteria, and any phage from our collection. Meanwhile, collection strain *Eam* K8, being different in FA content and plasmid replicon isolation, and suspected for being an R-variant or mutant, is the only one susceptible to phage infection.

Thus, isolated strains of *Erwinia amylovora* can be referred to as a highly conserved and homogeneous group that retains its features persisting in nature within a prolonged time. *Erwinia "horticola"* despite being closely-related are rather heterogeneous and significantly differ from *Eam*. Further investigation of underlying mechanisms that allow phages to infect *Eam* K8 and cause efficient lytic infection in it may shed the light on utility of phage-based anti-fire blight tools.