Identification of extracellular low-molecular-weight phosphonates of *Pectobacterium atrosepticum*

Parfirova O.I.^{1,2}, Gorshkov V.Y.^{1,2}, Tarasova N.B.¹, Gogoleva N.E.^{1,2}, Smolobochkin A.V.³, Petrova O.E.¹, Gogolev Y.V.^{1,2}

¹Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS", Kazan, Russia;

²Kazan (Volga region) Federal University, Kazan, Russia; ³A.E. Arbuzov Institute of Organic and Physical Chemistry, Federal Research Center "Kazan Scientific Center of RAS", Kazan, Russia

E-mail: parfirovaolga.i@gmail.com

Key message. Phytopathogenic *pectobacteria* produce extracellular low-molecular-weight phosphonates which possibly determine the strategy for the plant-microbe interaction.

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Phosphorus is one of the most important nature elements and plays an important role in many biological processes. One of the sources of phosphorus in nature is phosphonates - compounds whose characteristic feature is the presence of a carbon-phosphorus bond. According to various data, 10% of microorganisms contain genes encoding putative pathways of phosphonate biosynthesis, and about 40% of bacteria encode one or more pathways of phosphonate catabolism. The biosynthesis of phosphonates is diverse and common in nature, which suggests that the role of phosphonate molecules in the biosphere may be important. In particular, phosphonates are potent inhibitors of enzymatic activity and therefore may have antimicrobial and herbicidal properties.

By RNA-Seq analysis we have shown that *Pectobacterium atrosepticum* SCRI1043 (*Pba*) phosphonate-related genes are up-regulated during soft-rot development compared to latent infection or cultures *in vitro*. However, the phosphonates of phytopathogenic microorganisms are not described. The aim of this work was to establish whether phytopathogenic *pectobacteria* are capable of producing low-molecular-weight phosphonates and also to understand their physiological role.

To check if *Pba* produce extracellular low-molecular-weight phosphonates, *in vitro* conditions that induce the expression of phosphonate-related genes were selected. It was found that the expression of genes encoding enzymes for the synthesis of C-P bonds was increased in the presence of a plant extract. After culturing under these conditions, the supernatants were separated, and the expected low-molecular-weight phosphonates were extracted. NMR-spectroscopy was used for the detection of phosphonates in the supernatants of *Pba* cultures. ³¹P NMR-spectra for the obtained samples revealed the 21.91 ppm signal typical of phosphonates and the 59.69 ppm signal characteristic of phosphonium salts.

Locus-specific mutagenesis was applied to obtain mutant for the phosphonate biosynthesis-related genes. A *ΔfomI* (phosphoenolpyruvate phosphomutase is the main biosynthetic gene in this the metabolic way) deletion mutant did not produce these metabolites. But the signal of phosphonium salts was detected by ³¹P NMR. Perhaps this is due to different ways of biosynthesis of low molecular weight phosphonates and phosphonium salts.

Thus, we identified for the first time the extracellular low-molecular-weight phosphonates in plant pathogenic bacteria and obtained information on their role in pathogenesis. We are currently conducting a comparative analysis of the strategy of the interaction of plants with the wild and *ΔfomI* mutant forms of *Pectobacterium atrosepticum* and checking their virulence. We also perform the experiments of the determination of the molecular structure of the revealed compounds.

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