Features of the initial stages of the relationship between barley, phytopathogenic fungus *Fusarium culmorum* and rhizobacteria *Pseudomonas fluorescens* 2137

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Key message. It was shown that enhanced colonization of barley's roots by *Fusarium culmorum* in the presence of *Pseudomonas fluorescens* 2137 may be due to the composition of root exudates. Strain 2137 can enhance expression of plant defence gene *PAL*.

Keywords: *Fusarium culmorum*, *Pseudomonas fluorescens*, root exudates, root colonization

The development of effective methods for biocontrol of root rot requires a thorough study of the mechanisms of interaction between phytopathogenic fungi and beneficial rhizobacteria in the rhizosphere. Our studies are related to clarifying the question: why *Fusarium culmorum* in 1-4 days from seeds germination more actively colonized roots of barley *Hordeum vulgare* L. cv. Belogorsky that were colonized by *Pseudomonas fluorescens* 2137 (but not sterile roots), and what is the impact of this effect with the biocontrol activity of rhizobacteria?

The composition of root exudates and nutrition requirements of the *F. culmorum* and *P. fluorescens* 2137 was established using an ultra-performance liquid chromatography. The relationship between *F. culmorum* and antagonistic bacteria were studied in a growing experiment on membranes introduced into the rhizosphere, and also evaluated the relationship and quantity of each of the microorganisms on the roots of barley. The expression of *PAL* gene (one of the plant's protection genes) was determined by RT-PCR.

The absence of *F. culmorum* chemotaxis to root exudates of barley was established. However, it was found, that root exudates of barley colonized by *P. fluorescens* 2137 stimulated the growth of fungal growth tubes compared to exudates of uninoculated plants. A comparative analysis of low molecular weight components of root exudates showed that exudates of barley roots that were colonized by *P. fluorescens* 2137 contain a significantly higher amount of glucose and several amino acids (components to be necessary for the growth of *F. culmorum*), but lower amounts of aromatic acids (plant defense compounds having antimicrobial activity) than sterile barley root exudates or with colonization only *F. culmorum*. Stimulating the growth of *F. culmorum* could be the reason for more active colonization by the mycelium of the roots that were colonized by bacteria.

For experimental verification of the hypothesis of "extrusion" of a pathogen by antagonistic bacteria from the rhizosphere to the root surface, which leads to an increase in the amount of *F. culmorum* on the roots in the initial period of colonization, the relationship between the *F. culmorum* and the antagonistic bacteria *P. fluorescens* 2137, *Bacillus subtilis* Ch-13 and *Flavobacterium* sp. was studied. All bacteria used in this experiment suppressed the growth of *F. culmorum* mycelium in the barley rhizosphere. A comparison of the amount of fungus in the rhizosphere and on the roots in the presence of bacteria showed that an increase in the amount of fungus on the roots in the initial period of colonization was observed only in the presence of *P. fluorescens* 2137 and not observed in the presence of two other antagonistic strains. Thus, inhibition of the pathogen by antagonistic bacteria in the rhizosphere does not necessarily lead to an increase in the amount of *F. culmorum* on the roots in the initial period of colonization.

Similar nutritional needs in glucose and amino acids for *F. culmorum* and bacteria was led to competitive relationships between these microorganisms. Nevertheless, despite fluctuations in the number of each of the microorganisms on the roots as result of competition, the combined introduction of a pathogen and *P. fluorescens* 2137 into the substrate (vermiculite, soil) led to decrease in the intensity of root rot of barley, in compare with inoculation only *F. culmorum*. To clarify the mechanisms of root rot biocontrol, the expression of the *PAL* gene, one of the host protection genes, in sterile barley plants and colonized *F. culmorum* and *P. fluorescens* 2137 were assessed. In daily barley plants, the strongest expression of *PAL* was noted in roots colonized only by *P. fluorescens* 2137, and in three-day-old barley plants the expression level of this gene increased in roots colonized jointly by the pathogen and *P. fluorescens* 2137. The observed response of barley to the colonization of *F. culmorum* and *P. fluorescens* 2137 indicates that strain 2137 may cause a more active protective response in barley than a phytopathogenic fungus. It is possible that in the early stages of the relationship between *F. culmorum*, *P. fluorescens* 2137 and barley, the role of rhizobacteria in control of root rot is mainly associated not with the direct suppression of *F. culmorum* on the roots (which was not observed in our experiments), but with early induction protective reactions of barley.

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