Plant bioactive compounds inducing stress response in pathogenic fungus Fusarium proliferatum

The principal scientific aim of the project is to identify the metabolites present in the plant extracts prepared from tissues of the pathogen's host species that may be involved in the induction of the stress response in plant pathogenic Fusarium proliferatum strains.

Fusarium species are among the most common fungal plant pathogens. They are able to cause multiple diseases reducing yield and quality of many crop species worldwide. Virtually all Fusarium species are able to synthesize toxic secondary metabolites, known as mycotoxins or fusariotoxins and three groups of those metabolites are among the most dangerous contaminants of cereal grain: deoxynivalenol, zearalenone and fumonisins. The B group analogues of fumonisins are the most abundant mycotoxins synthesized by Fusarium proliferatum both in vitro and in plant tissues of plant host species, which may be: maize, rice, wheat, peas, soybean, pineapple, date palm, asparagus, garlic, onion, fig, banana and many others. Since the range of hosts of this pathogen is wider than for most of the Fusarium pathogens, it seems likely that F. proliferatum has evolved some form of advantage plant during infection process. To some extent also a broad range of synthesized mycotoxins support this hypothesis. To investigate deeply this phenomenon, five extracts prepared from the tissues of plant species commonly hosting F. proliferatum pathogen (maize, asparagus, garlic, pineapple and peas) were used to evaluate the influence of the extracts on pathogen's metabolism.

Consistent patterns of changes in fungal biomass and fumonisin biosynthesis were recorded for individual extracts studied. Fumonisin concentrations in culture media increased shortly after extracts were added and decreased afterwards, indicating that extract addition moment could be regarded as a stress factor for the pathogen. This hypothesis has been well supported by the differential proteomic analysis, which has indicated that stress response proteins are induced after host extracts addition. Very recently, it has been proven that Fusarium pathogens possess similar signal-transducing pathways to those present in plants. As a consequence, in vitro studies of Fusarium reaction to various stresses (e.g. salinity, heat, cold, light or water stress) have gotten in the current research more and more attention. Therefore, we decided to investigate the molecular interactions between Fusarium proliferatum pathogen and its host, represented by the molecules present in each of five extracts added to the liquid culture of the fungus.

To our knowledge, the approach to look for plant stress inducers in fungal pathogens has never been used before. On the contrary, it’s the plant stress response pathways have been studied widely in model and crop species. In this proposal we are focusing on identification of plant metabolites present in each of five plant extracts, able to induce stress response in two Fusarium proliferatum strains of different host origin. Only water-soluble metabolites will be analyzed, since they proved to exert specific effects in previous research. Independently, stress response to extreme temperatures (35°C and 10°C) and salinity (NaCl) will be evaluated in the same strains, to conclude whether similar mechanisms are induced by various types of stress factors. Having proven a specific reaction to plant metabolites present in the extracts, it would be possible to point out the metabolic pathways involved in the plant-pathogen interaction.

Some of the plant compounds have already been assigned to have specific functions in altering the fungal metabolism. The best known example is amylopectin, one of the basic starch ingredients, stimulating the biosynthesis of fumonisins. Also the influence of other sugars and certain amino acids on in vitro fumonisin biosynthesis has been characterized. However, many other compounds of plant origin, being recognized by F. proliferatum as molecular “switches” of specific metabolic pathways, remains still to be discovered.

Experiments covered by the proposed project can provide evidence for specific effect of certain plant bioactive molecules present in the specific host extract fractions, able to induce stress response in F. proliferatum strains. Extract fractions inducing actively stress response in the pathogen will be identified using mass spectrometric analyses. During previous project we were able to identify at least 100 proteins induced by the host extracts by the MS analysis of the differentially expressed peptides separated using 2D-gel electrophoresis. Of all identified proteins, a number appeared as stress response-related ones. Actually none of the proteins was yet analyzed during this type of stress applied to the pathogenic fungus. Therefore, this approach appears as unique opportunity to assign new roles and functions to pre-defined peptides and enzymes: heat shock proteins, dismutases, kinases, amino acid synthases, methylotransferases and others. Transcript levels of the genes encoding those proteins will be studied during cultivation of the stressed cultures of the pathogen strains. The expression of the fumonisin biosynthetic gene FUM1 will also be analyzed, as the transcript levels of this gene was found to be highly increased after exposure of the fungus to some of the host extracts.

Plant response to biotic and abiotic stresses and mechanisms underlying this response has been widely studied for many years, both using model (Arabidopsis) and crop plant species, just to mention oilseed rape, wheat, maize and tomato. Fungal metabolites, responsible for plant stress reaction, have also been tested along with the biochemical pathways of those metabolites' biosynthesis. On the other hand, relatively little is known about how the pathogen and its host plant organisms communicate on molecular level. Recently, involvement of several kinases in signal transduction and multiple stress response in Fusarium graminearum has been reported. This pathogen is the most known worldwide, and it is very likely, that more research will be published soon on other common Fusarium pathogens with known full-genome sequences and well-established research methodology: F. oxysporum, F. verticillioides and, possibly, F. fujikuroi. However, none of the published studies was focused on stress-inductors of plant origin, neither involved F. proliferatum as a model. In our opinion, these two decent advantages make the proposed project novel and exceptional.