Volume 58(1):73-94, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Investigation of South-Indian *Fusarium* isolates from human keratitis

Mónika Homa

Department of Microbiology, University of Szeged, Szeged, Hungary

The genus *Fusarium* is a large group of hyaline filamentous fungi. They are widely distributed in soil as harmless, saprophytic organisms. However, some members of this genus are capable of causing infection in plants, animals and humans. *Fusarium* spp. are the most frequently isolated causative agents of human keratomycosis in South India. Antifungal susceptibilities of different *Fusarium* species complexes (SCs) vary, and members of the *F. solani* SC (FSSC) show remarkable resistance to most clinically applied antifungal drugs. Thus the misidentification of the causative agent and the subsequent application of an inappropriate antifungal therapy could result in the loss of vision. Using molecular techniques in laboratory practice instead of conventional morphological methods can make the identification process more accurate and faster. New antifungals and alternative treatments would also be appropriate to prevent or treat the infection.

For these reasons, first we identified *Fusarium* strains isolated from human keratomycosis at the Aravind Eye Hospital and Postgraduate Institute of Ophthalmology (Coimbatore, India) in the years 2004-2005 and 2010-2011 using different molecular methods. We also examined the SC diversity between the two sampling periods. Our results indicate that the members of the FSSC are the most frequently isolated species from keratomycosis in South India, and the incidence of the less frequent human pathogenic *Fusarium* species seems to be increasing.

We also determined and compared the antifungal susceptibilities of the previously mentioned strains. Natamycin (NTM) proved to be the most effective drug against the tested isolates, followed by amphotericin B and terbinafine (TRB). Changes in the minimal inhibitory concentration (MIC) values of NTM and TRB were not observed between the isolates derived from the two sampling periods, but the *in vitro* susceptibility to azoles decreased up to 2011. NTM and TRB were also applied in antifungal combination susceptibility tests because of their high *in vitro* efficacy and their differing antifungal mechanisms. These compounds together showed a similar or a better antifungal activity on Fusaria than each of the compounds alone, as they could interact synergistically.

As a potential alternative cure for the infection, we examined the *in vitro* inhibitory effect of 9 different essential oils on 18 *Fusarium* strains isolated from keratitis. The lowest MICs were observed in the case of *Cinnamomum zeylanicum* oil; and its component, trans-cinnamaldehyde (tCA) was also tested and showed the same activity against the investigated isolates. The *in vitro* interaction between tCA and NTM was also determined. Furthermore, we investigated the antifungal mechanism of cinnamon oil and tCA by microscopic observations. Based on these observations both the oil and its component caused delayed or inhibited germination of conidia and reduced cellular metabolism. Thus, they can be potentially used in the treatment of *Fusarium* keratitis. However, the preliminary *in vitro* studies suggest that their simultaneous application with antifungal drugs, such as NTM, will not increase the efficacy of the therapy.

The investigation of phylogenetic relationships among clinical and environmental isolates and the production of extracellular enzymes, as potential virulence factors, are in progress.

The research of M.H. was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'. The relating research groups were also supported by the INSA-HAS interacademic bilateral project (SNK-49/2013) providing infrastructure and research equipment.

Supervisors: László Galgóczi, László Kredics
E-mail: homamoni@gmail.com

Up-regulation of defense genes in pepper leaves inoculated with tobamoviruses

Csilla Juhász

Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Virus infections result in substantial alterations of gene expression patterns in infected plant tissues including the up-regulation of a wide variety of defense-related genes. These defense reactions are controlled by a complex, multilayered regulatory network in which various transcription factors and defense-related plant hormones play critical roles. In addition, host intracellular membrane lipids also substantially influence virus replication. Upon infection, tobamoviruses induce substantial modifications in intracellular host membranes in order to create protected viral replication compartments. During this process the structure of membrane lipid bilayers is substantially modified. Viral RNA synthesis is highly sensitive to lipid composition and particularly to the level of unsaturated fatty acids.

In recent years our research has been focused on the defense reactions of pepper (*Capsicum annuum* L.) plants following virus inoculations. We have used two different viruses in order to compare compatible and incompatible pepper-virus interactions. Inoculation with *Obuda pepper virus* (ObPV) led to the appearance of hypersensitive necrotic lesions on the inoculated leaves. In contrast, very mild symptoms appeared on the leaves inoculated with *Pepper mild mottle virus* (PMMoV). Although these plants seem to be healthy, the virus is spreading from the infection site into the whole plant causing very serious stunting and the pepper fruits will be very strongly distorted.

Volume 58(1):73-94, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ObPV-inoculation resulted in the marked up-regulation of genes encoding PR-proteins, a patatin-like lipase (lipid acil hydrolase), a defensin, a 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and a dioxygenase participating in carotenoid degradation. In addition, ObPV-inoculation led to a rapid and massive up-regulation of several individual 9-lipoxygenase (*9-LOX*) genes. In contrast, *13-LOX* genes were only moderately induced by ObPV. The expression of several genes encoding WRKY transcription factors were also induced by ObPV. In contrast, the expression of defense genes increased in most cases to a lesser extent in PMMoV-inoculated, susceptible leaves or in mock-inoculated leaves. Plant hormones and an ethylene precursor (salicylic acid, methyl-jasmonate, and ACC) induced very differently the expression of individual *LOX* and *WRKY* genes.

In summary, our results showed that the rapid and massive up-regulation of defense genes encoding PR-proteins, LOXs and WRKY transcription factors in the incompatible pepper-ObPV interaction contributes to antiviral resistance. We suppose that by the rapid up-regulation of *9-LOX* genes pepper plants are able to alter the structure of intracellular membranes in order to inhibit the replication of invading tobamoviruses.

Supervisor: Gábor Gullner
E-mail: juhasz.csilla@agrar.mta.hu

Investigation of the different mechanisms of the innate immune response of *Drosophila melanogaster*

Beáta Kari

Immunology Unit, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Drosophila melanogaster has been widely used model organism to study host response to microbial and parasitic infections. The chitin cuticle of the adult *Drosophila* is the first barrier against microbial invasion. Injury of the cuticle activates hemolymph clotting, which blocks the loss of body fluids and the spreading of the microorganisms into the hemocoel by immobilizing bacteria at the wound site. Pathogens entering the hemocoel activate both cell-mediated and humoral immune responses. The cell-mediated arm of the immune response is carried out by the hemocytes, the production of antimicrobial peptides are regulated by the Toll and the immune deficiency (Imd) pathways.

We developed and validated a new method to identify novel factors involved in the hemolymph coagulation and in the host-pathogen interactions after septic injury.

The method, based on inducing lesion by removing the tarsal segments of the first pair of legs of *Drosophila* adults and exposing them to different bacteria, imitates injury that often occurs in the natural habitat. The technique was validated by using mutant variations of different components of the immune response; blood clotting as well as the involvement of a number of genes known to be instrumental in the humoral and cell-mediated immune responses of *Drosophila* was confirmed. We used the slightly pathogenic *E. coli*, the semi-pathogenic *B. cereus* and the highly pathogenic *S. marcescens* and monitored the viability of the flies. First, we tested the survival of the control w^{1118} and mutant flies after sterile injury and the survival of the non-injured w^{1118} and mutant lines (spz^2/spz^4, $Dredd^{EP1412}$, Rel^{20} and Hml^{f03374}) treated with *E. coli, B. cereus* and *S. marcescens*. We found that the survival of non-injured mutant flies treated with *E. coli, B. cereus* and *S. marcescens* were similar. The injury itself do not affect the survival of the animals, except for the Hml^{f03374} homozygotes, which lose more hemolymph after wounding and showed decreased survival rate following both sterile and septic injury compared to the control. We found that the Imd pathway mutants $Dredd^{EP1412}$ and Rel^{E20} and the hemolymph clotting factor *Hemolectin* (Hml^{f03374}) mutant flies showed reduced viability after either *B. cereus* or *E. coli* infection, while the *spätzle* (spz^2/spz^4), involved in the Toll pathway, was significantly sensitive to *B. cereus* infection. By using this novel method, we have found that the *raspberry* gene is involved in the survival of the fly after septic injury, since the mutants have decreased survival rate after *B. cereus* infection. This gene encodes the *Drosophila* inosine monophosphate dehydrogenase, and is a key enzyme of the *de novo* synthesis of guanine nucleotides. In mammals, *de novo* GMP synthesis is required for lymphocyte proliferation and in the immune response. We will study the function of the *raspberry* in the immune response of the *Drosophila*.

Our new method is suitable for high-scale screening of key factors involved in host-pathogen interactions following a septic injury. It also offers an alternative to previous experiments, where microinjection needle were used to administer microbes into the body cavity. A major advantage of this method is that the wound by itself is insignificant, the effect on survival can be attributed entirely to the infection and the defensive capabilities of the host organism.

Furthermore, we identified a new marker molecule 3A5 in the cytoplasm of a subset of plasmatocytes in all hematopoietic compartments, in the circulation, in the lymph gland and in the sessile tissue and in the hemolymph. We study the function of 3A5 molecule in the *Drosophila* immune response and in the coagulation reaction.

Supervisor: Éva Kurucz
E-mail: kari.beata@brc.mta.hu