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

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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.

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

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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.