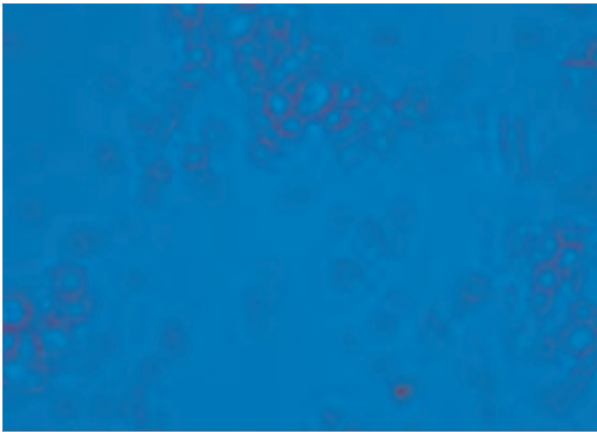
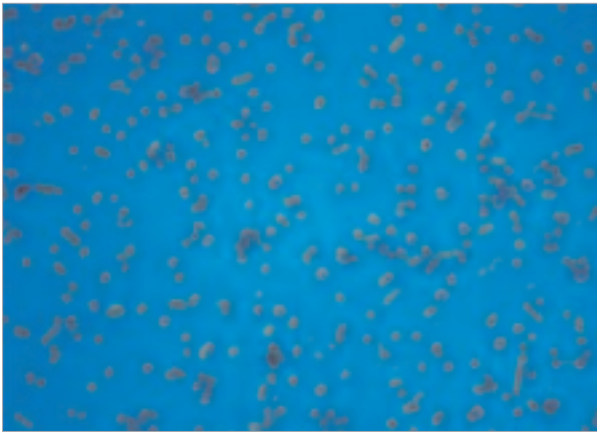


MOLECULAR GENETICS AND FUNCTIONAL GENOMICS OF FUNGI

Nilce Maria Martinez ROSSI

Ribeirão Preto Medicine School / University of São Paulo (USP)



*Microphotographs of microconidia (a)
and arthroconidia (b) of Trichophyton sp.*

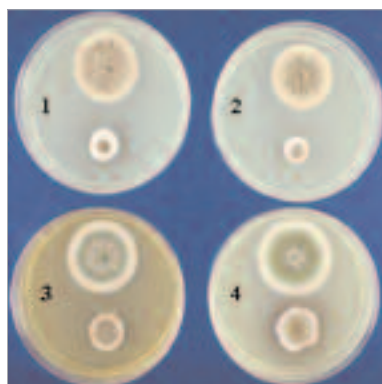
Dermatophytosis are superficial mycoses caused by a group of filamentous fungi called dermatophytes, which are able to invade keratinized substrates such as hair, nails and stratum corneum. *Trichophyton rubrum*, an anthropophilic and cosmopolitan fungus, is the most common agent of superficial mycoses, causing rarely deep dermatophytosis in immunocompromised hosts.

The regulation of gene expression is vital for all organisms and is especially required by fungi for rapid adaptation to cellular stress conditions, as is the case when they infect a host or when they are submitted to antifungal drugs. These adaptive mechanisms are extremely complex and most of them have not been fully clarified. The objective of the present project is to elucidate the metabolic pathways or cellular processes that permit fungi to survive under adverse conditions. In addition, we intend to identify genes that may be involved in pathogenicity and that therefore may become targets for the development of new antifungal drugs.

A determinant factor in the invasion and utilization of host tissue by fungi is the secretion of enzymes, which is frequently regulated by ambient pH. Consequently, the mechanisms of pathogenicity or even of resistance to inhibitors are likely to depend directly or indirectly on ambient pH monitoring. Thus, knowledge of the mechanisms that regulate the expression/secretion of enzymes involved in pathogenicity and of enzymes responsible for resistance to inhibitors will be of fundamental importance in the search for new therapeutic strategies, in the revelation of new drug targets, and therefore in the control of microorganisms that are harmful to the man.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The fungi regulate many cellular events as a function of pH environment. This genetic mechanism has extensive practical application, including installation, development and maintenance of dermatophytes in the host. Genes preferentially expressed in pH acid were identified in *T. rubrum*, a condition which mimics that found in the human skin. We show that the secretion of queratinases (virulence factor) by *T. rubrum* is dependent on the gene *pacC*, because the knockout of this gene promoted the decreased secretion of these enzymes, affecting growth in fragments of human fingernail. In addition, we revealed three



Colonial growth of the model fungus *Aspergillus nidulans* showing phenotype changes dependent on the composition and pH of the culture medium. (http://www.fgsc.net/home_page_image_information.htm)

molecular mechanisms of terbinafine resistance in fungi, an antifungal widely used for the treatment of dermatophytosis: a) the presence of multiple copies of a gene (*salA*) accelerates the metabolism of terbinafine; b) mutations in the squalene epoxidase gene cause resistance to this drug because it prevents the binding of terbinafine to squalene epoxidase, its target cell, and c) a transporter protein of *T. rubrum* participates in the terbinafine efflux. Our laboratory has also focused on the response to

the stress caused by other drugs used to treat human mycoses, which have revealed new genes in dermatophytes, some of them potential targets for the development of new antifungal agents.

Several methods have been standardized to study *T. rubrum* such as the electrophoretic molecular karyotype, the molecular transformation and gene knockout that will allow the functional analysis of virtually any gene of this dermatophyte. As a result of these findings, the group was invited to join the Steering Committee of the dermatophytes genome project developed by the consortium formed by The Broad Institute of Harvard and MIT and the NIH (National Institute of Health). The group was also invited by the editor of the journal *Mycopathologia* (Springer) to write a review about Antifungal resistance mechanisms in dermatophytes (DOI 10.1007/s11046-008-9110-7).

MAIN PUBLICATIONS

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Nilce Maria Martinez ROSSI

Faculdade de Medicina de Ribeirão Preto
Universidade de São Paulo (USP)
Avenida Bandeirantes, 3900 – Monte Alegre
CEP 14049-900 – Ribeirão Preto, SP – Brasil
+55-16-3602-3150
nmmrossi@usp.br