

Habilitation thesis

INNOVATIVE GENETIC, TAXONOMIC and METABOLOMIC STUDIES on YEASTS

with THEORETICAL, BIOTECHNOLOGICAL and INDUSTRIAL IMPORTANCE

- Abstract -

The professional, academic and scientific work presented in the habilitation thesis with the title “*Innovative genetic, taxonomic and metabolomic studies on yeasts with theoretical, biotechnological and industrial importance*” in the domain of **Biology**, expands over 14 years, after acquiring the PhD title in 2002 (2002 - 2016). My activity at the University of Bucharest, Department of Genetics, began 21 years ago, in 1995, as a research assistant. From 1998 I was an assistant professor, lecturer, associate professor and, from 2015 until present, I am a professor. My academic activity consists in courses and leading lab sessions for undergraduated and Master students and for specialists (POSDRU project 81/3.2/S/55362) in the domains: *Genetics, Microbial genetics, Molecular genetics and genetic engineering, Genetics and taxonomy of microorganisms of biotechnological importance, Molecular techniques for microorganisms with biotechnological applications, Molecular mechanisms of pathogenity and virulence*. I was also involved in collaborations and acted as technical advisor for Ph.D. students.

After delivering my PhD thesis in 2002, I participated in 19 national projects and grants, for 2 of them as project director (CNCSIS 1668/2007; UEFISCDI-IDEI 985/2009). The most important scientific contributions and the specific research domains approached during my activity are presented in *Part I: Professional, academic and scientific achievements*.

The yeasts have been related to human activity since the begining of the evolution of the human society, being involved in main domains of industry (food, beverages, chemicals, pharmaceuticals) and biotechnology (bioremediation, biocontrol, biomedicine). Moreover, due to their genetic structure and regulation processes highly similar to those from mammalian cells, the yeasts represent optimal models for theoretical studies aimed to provide information used in therapeutics and medicine. Therefore, there is an increasing need for the characterization of novel yeast strains and species isolated from natural and / or industrial environments. **Chapter I.2.1.** presents the *research directions and accomplishments in the domain of yeast taxonomical identification, biodiversity and genetics*. On this purpose, during a number of research projects and the academic activity with the students, a wide range of molecular techniques were developed and optimized, establishing species and strain

specific parameters for the accurate characterization of the nuclear and extranuclear genome of novel yeast strains. The phylogenetic analyses offered new insights on the biodiversity of yeasts with practical applications, isolated from various environments. The studies performed during my international postdoctoral stages, in collaboration with scientists from the domain, represented the basis for physical and genetic mapping of *Kluyveromyces lactis*, an yeast species frequently used in food industry and for probiotic compounds. Also, important results were obtained on the regulation of genes involved in DNA damage and repair, with future implications for studies regarding some human disorders.

Chapter I.2.2. focuses on *biochemical and metabolomic studies of yeasts of high industrial, biotechnological and biomedical interest*. The industrial development and the progress of new urban communities, raised the problem of storage and treatment of augmented quantities of wastes and oil spills, for preserving the nature. *Bioremediation* has a minimal environmental impact representing an ecological remediation technology. Our work on yeast species from *Candida*, *Issatchenkia*, *Rhodotorula* and *Yarrowia* genera resulted in new data concerning the ability of yeasts to degrade polluting compounds and in the identification of the mechanisms of hydrocarbon assimilation in the cell. An interesting aspect of yeasts with biodegrading abilities is represented by the synthesis of biosurfactants intensively used in chemical and food industries, cosmetics and medicine. During our research, we established and optimized the laboratory conditions and methods for biosurfactants obtaining using low cost substrates such as industrial (hydrocarbons, petroleum, glycerol) and household wastes (vegetable oils), with high practical potential both at national and international level. Stability and antimicrobial studies allowed the characterization of the obtained biosurfactants. We also determined and described a possible correlation between biosurfactant production and the synthesis of lipases and lipids in *Candida* cells.

Yeasts with antimicrobial activities are intensively used in *biocontrol technologies* aimed to protect fresh vegetables and fruits against phytopatogens, as an alternative to chemical treatment with fungicides and other compounds with highly toxic potential for human and animal health. Our studies on *Metschnikowia pulcherrima* analyzed the influence of the culture conditions and revealed the specific mechanism of the antagonistic activity. New strategies were studied and developed for the augmentation of the antimicrobial activity against human pathogenic *Candida* strains, with future *biomedical applications*. Also, studies were performed regarding the killer activity of yeasts belonging to *Kluyveromyces* and *Saccharomyces* genera, and we analysed the mechanism of action of the killer toxin using a wide range of strains with industrial and medical importance.

During last decades, the international scientific community shows a growing interest in the ***production of yeast lipases*** able to catalyze a wide range of chemical reactions being thus used in bioremediation and bomedicine, for obtaining biofuels, detergents, chemical and pharmaceutical compounds. During our studies we selected new lipase producing yeast strains belonging to *Candida*, *Yarrowia*, *Saccharomyces*, *Kluyveromyces* and *Rhodotorula* genera. The optimal parameters for the induction of the lipolytic activity were established, and the effect of various substrates on lipase synthesis was determined. Also, a strain-specific correlation was observed between the carbon source from the environment and the dimorphism of *Candida* yeasts.

The last section of the Chapter I.2.2. deals with the ***genetic improvement of methylotrophic yeast strains***, for obtaining high rates of methanol assimilation and biomass production. Some of the improved strains were auxotrophic, representing the basis for further studies aimed to develop cloning / expression vectors with biotechnological applications.

The scientific work presented in the thesis is based on original studies financed by national research projects. Along my activity, I was main / co-author for ***106 publications: 4 books - 3 books after the PhD thesis, for 2 as unique author; articles published in ISI (23) and international data bases indexed journal (29), in other journals (3), one patent*** and participations at ***national and international scientific events (46)***, with ***250 citations and Hirsh index (h) 7***. Also, I am ***reviewer for 9 ISI or IDB indexed journals*** and ***member of the Editorial Board (Associated Editor) for the journal Romanian Biotechnological Letters (ISI indexed)***.

Part II: Career perspectives, begins with ***II.1. Future professional and academic developments***, which aims to augment the scientific level and efficiency of the teaching activity and to establish, in collaboration with other specialists, the content of the training curriculum for undergraduate and Master students in biology and biochemistry, in order to assure their employment according to the professional standards of the European Union. Also, in collaborations with my colleagues I am preparing two books comprising theoretical information and laboratory techniques in the domains of microbial genetics and biotechnology.

Chapter II.1. presents the ***Future research directions***: (a) ***development of the microbial collection of the Department of Genetics by including new and fully characterized yeast strains with theoretical and practical applications*** (isolation and accurate identification of novel strains, creation of a database comprising the molecular features of the strains, innovative approaches for biodiversity studies and phylogenetic cluster analyses, establishing specific molecular markers for rapid identification of yeasts from various

environments), starting the process of *accreditation of the collection* according to the requirements of European Culture Collections' Organisation (ECCO) and World Federation of Culture Collections (WFCC); (b) *molecular studies on the biodegradation pathways of various pollutants with increased risks for the environment and human health* (insights on gene regulation processes involved in hydrocarbon degradation, development of strategies for biosurfactants production and applicability and for phytoremediation of polluted and agricultural soils); (c) *elaborate genetics and physiology studies on yeasts with antimicrobial potential* (elucidation of antimicrobial mechanisms, obtaining bioproducts with applications in food industry, agriculture and biomedicine).

In order to continue my scientific work and to achieve the proposed research goals, I am involved in numerous present project proposals and I will continue to apply in future national and international grant competitions.

The last chapter of the thesis, *Part III. References* comprises 297 titles, including **52 publications presented in the thesis, for which I am main or co-author.**

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Prof. dr. Csutak Ortansa Elisabeta