CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRALOVE

Department: Department of Biological and Medical Sciences

Master´s degree program in Pharmacy

Opponent´s review of Master´s thesis

Student´s name: Kollárová Nikola

Mentor of the thesis: Prof. Gloria Molero, Prof. Lucia Monteoliva, PharmDr. Ondřej Janďourek, PhD.

Year of the thesis defense: 2017

Opponent of the thesis: RNDr. Klára Konečná, PhD.

Title of the thesis:
Write or insert : Detection of Sap2 in the secretome of Candida albicans cell wall and secretory mutants

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Formal comments: number of pages: 67, number of figures: 26, number of tables: 10, number of references: 72.

Type of work: Experimental work

a) The aim of the thesis is: Fulfilled

b) Language and graphic level: Excellent

c) Processing of the theory: Excellent

d) Methods description: Very good

e) Results description: Excellent

f) Discussion and conclusions: Excellent

I recommend Diploma thesis for the recognition as Rigorous thesis ☒.

Opponent´s comments:
At the page 13, " C. albicans is aerobic and …", I have to disagree with this formulation. C. albicans is capable to metabolize different substrates in anaerobic condition, as well. It is facultative anaerobe.
At the page 14, " caustic mounts …." author means KOH mounts. The designation "caustic mounts" is not usually used for wet KOH mounts of yeasts/molds.
At the page 22, Figure 6.: explanation of all shortcuts in the Figures (mosty bellow figures), in this case the shortcut Hsps is recommended.
At the page 23, there is an information: "Invasins, such as E-cadherin on epithelian cells …." Invasins are in this case pathogen molecules (virulence factors, important for pathogenesis, not host molecule E-cadherin). Author describes, that there are two invasins of Candida albicans: Als1 and Ssa1. Mostly, Als3 is described to be the key adhesin/invasin of Candida albicans. Is the role of Als1 the same to Als3?

At the page 29, author describes unconventional/alternative ways of effector molecules secretion and refers to Figure 18. In fact, in Figure 18, there is only scheme with classical secretory pathway/classical secretion, unconventional secretion pathways are there not represent.
At the page 30, the list of chemicals and media; it is recommended to specify selling company/product name of all chemicals, purity etc. E.g. in case of east nitrogen base, there are commercially avalilable different products with different chemical composition.
Some chemicals, instruments are missing in the list of chemicals/instruments, e.g. protein marker, Odyssey CLx Imaging System, etc.
At the page 34, author refers to Figure 11, instead of Figure 10 - plates with U-bottom to see compact pellets.
At the page 36, there is formulation: " Preparation of protein extracts from culture supernatans and cytoplasmic extract ….". Mostly cytosolic, membrane protein extraction (chemical, physical extraction), etc. is done, not protein extraction from supernatans. Supernatant are mostly used for the analysis of the secretome, protein concentration and purification is mostly done, but the term protein extraction is not usually used.
At the page 43, author refers to Figure 16, instead of Figure 15 - SDS-PAGE electrophoresis.
At the page 49 (51), author refers to Figure 24 (25), instead of Figure 23 (25).
At the page 52, there is a mistake in Sap designation …. "other members of the same family (Sap3, 7, , 9 and 99).
Conclusion: Although, there are some slight formal imperfections in this thesis, the topic, information processing in the theoretical part, results description and especially result discussion and conclusion of this thesis is in high quality level.

Questions:
1) Are chlamydospores produced by all Candida albicans strains? Describe chlamydospore in more details. How can we induce chlamydospores formation in this yeast?
2) Summarize factors affecting the switch of Candida albicans from yeast form to hyphae.
3) Is the virulence factor of Candida albicans, Sap2, expressed constitutively or there is induced expresion of the gene coding this protein? Why the temperature 30 Celsium degrees was used for the study? Mostly, the temperature of 37 Celsium degrees (host temperature) is limiting for the secretion of virulence factors of many microbial pathogens …

Evaluation of Master´s thesis: Excellent

Recommendations for the thesis defense: Recommended

In Hradec Kralove 24.5.2017 ……
Opponent´s signature