The protective potential of *L. rhamnosus* is highly *C. albicans* strain-dependent. Our data hint toward a potential multifactorial effect involving stress-resistance and metabolic interplay. Elucidating the processes that lead to epithelial protection or enhanced damage will be crucial to predict whether probiotic lactobacilli may be beneficial or detrimental for a patient and may help to design generally protective probiotics.

P343

Novel hydrophobic binding surface proteins are instrumental for phagocytosis of *Lichtheimia corymbifera* by macrophages

Jaime David Acosta España[1,2], Mohamed Abdelwahab Hassan[1,2], Park Hea-Reung[1,2], Volker U. Schwartze[1,2], Sascha Schäuble[1,2], Thomas Krüger[1], Olaf Kniemeyer[1], Axel A. Brakhage[1,2], Kerstin Voigt[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany
[2]Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University Jena, Jena, Germany

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives:

- Perform proteomic analysis of the spore surface alongside secretome analysis of two strains of *Lichtheimia corymbifera*.
- Evaluate interaction, recognition, and phagocytosis of *Lichtheimia* spores by murine alveolar macrophages.

Methods: Two strains of *L. corymbifera* (JMRC: FSU: 09682 and JMRC: FSU: 10164) were used in this study. For phagocytosis assay, the spores were labeled with 0.1 mg/ml Fluorescein isothiocyanate (FITC) (Sigma Aldrich Chemie) in 0.1 M Na2CO3 for 30 min 146 at 30°C. Murine alveolar macrophages MH-S (ATCC:CRL-2019) were cultivated in RPMI-1640 (Sigma, 30-2001) supplemented.

Identification of the surface proteins of *L. corymbifera* was carried out as described before with minor modification.[1] The supernatants were stored at −80°C for liquid chromatography-mass spectrometry (LC-MS/MS) analysis. Secreted proteins of *L. corymbifera* were determined as described in a previous study with minor modification[2].

The raw files generated by the LC-MS/MS were further processed by the software Proteome Discoverer v1.4.0.288 (Thermo). Tandem mass spectra were searched against the NCBI *L. corymbifera* protein database.[3] Approximately 10 000 MH-S cells were cultivated onto 96-well microplates (NUNC 163320) in 100 μL RPMI-1640 medium (Sigma, 30-2001). Images were acquired by the Zeiss Axio Observer 7 Spinning Disk Confocal Microscope (ZEISS, Jena, Germany) and processed with ZEN 2.1 Software (ZEISS) by 63x objective lens.

Results: Abundant surface proteins were found which serve as Candidates for secretome analysis. A total of 113 proteins were identified. Thirty proteins were confirmed to be on the spore surface based on the presence of signal peptides[3]. The following proteins were predominantly identified: Spore coat protein (CotH), hydrophobic surface binding protein A (HsbA), aconitase, ricin-like lectin, two transition elongation factors, multi-copper oxidase, heat shock protein 70 family (Hsp70), malate synthase, putative allergen Candidates, etc.

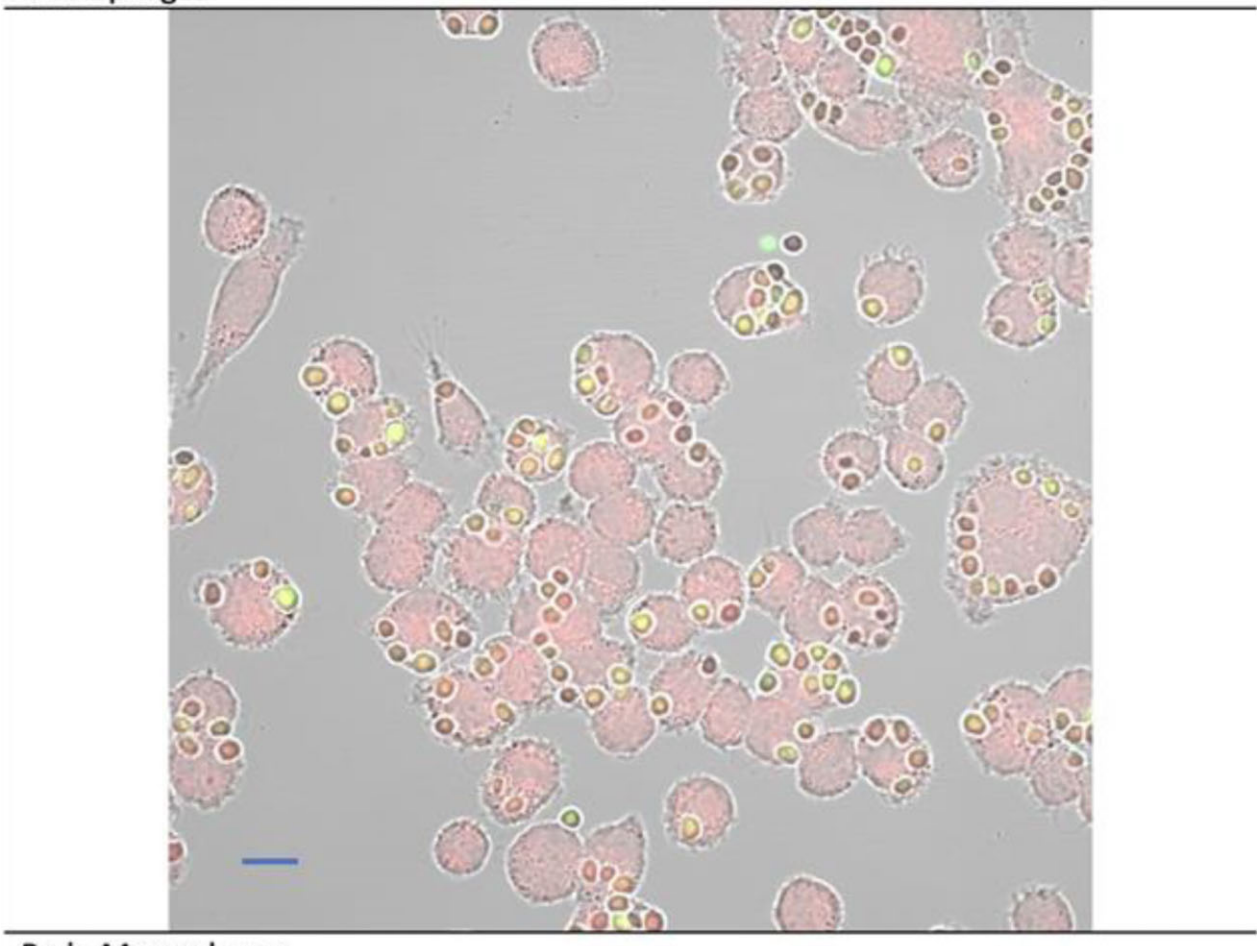
These proteins were heterologsly overexpressed in yeast. Successful overexpression was confirmed by LC-MS/MS. The yeast mutants were subjected to phagocytosis assays (Fig. 1). The role of surface proteins in macrophages is discussed.

Conclusion: The surface proteins are instrumental for recognition of *L. corymbifera* by macrophages and for intracellular survival in macrophages. The role of surface proteins is discussed in the light of evolution from environmental to human pathogenic fungus.

Sources:

1. Voltersen V, Blango MG, Herrmann S, et al. Proteome Analysis Reveals the Conidial Surface Protein CcpA Essential for Virulence of the Pathogenic Fungus *Aspergillus fumigatus*. mBio. 2018;9(5). doi:10.1128/MBIO.01557-18
2. Thürich J, Meichsner D, Furch ACU, et al. Arabidopsis thaliana responds to colonisation of Piriformospora indica by secretion of symbiosis-specific proteins. *PLOS ONE*. 2018;13(12):e0209658. doi:10.1371/JOURNAL.PONE.0209658.
3. Schwartze VU, Winter S, Shelest E, et al. Gene Expansion Shapes Genome Architecture in the Human Pathogen Lichtheimia corymbifera: An Evolutionary Genomics Analysis in the Ancient Terrestrial Mucorales (Mucoromycotina). *PLOS Genetics*. 2014;10(8):e1004496. doi:10.1371/JOURNAL.PGEN.1004496.

Figure 1.- Phagocytosis of heat-killed *L. corymbifera* spores by murine alveolar macrophages



Red.- Macrophages
Green: *L. corymbifera* spores Scale bar in blue: 10µm
Green *L. corymbifera* spore are not acidifying and spores in red are acidifying
Technique: confocal laser scanning microscopy using LysoTracker dye