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Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute

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Kaswara Kraibooj^{1,2}, Hea-Reung Park^{2,3}, Hans-Martin Dahse⁴, Christine Skerka^{2,4}, Hanno Schöler^{2,5}, Kerstin Voigt^{2,3}, Axel Brakhage^{2,5} and Marc Thilo Figge^{1,2}

¹Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany
²Freiburger Institute für Systemische Biologie, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany
³Jena Molecular Resource Collection, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany
⁴Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany
⁵Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany

1. Introduction

- Motivation:** Immunocompromised patients are susceptible for opportunistic infections of human-pathogenic fungi.
- Goal:** Quantification of host-pathogen interactions in comparative studies of different mutants.
- Methods:**
 - Resting biological questions → How do immune cells interact with human-pathogenic fungi?
 - Combination assays → Preparing cell cultures
 - Imaging → Producing a sufficient number of images using confocal or fluorescence microscopy
 - Image analysis → Pre-processing, segmentation and classification
 - Statistical analysis → Computing characteristic quantities like phagocytosis and adhesion index
 - Biological interpretation → Better understanding of the infection process

2. Example: Phagocytosis Assay

An automated analysis of 360 fluorescence microscopy images is performed [1]. The analysis is based on the method applied by Mech et al. [2]. The images depict the interaction between *Lichtheimia corymbifera* and murine alveolar macrophages. A virulent (JMRC-FSU-3662) and an attenuated (JMRC-FSU-10164) strain of this fungus are studied under three conditions: resting, swollen and opsonized spores.

- The algorithm is developed using the software environment Definiens®.
- We computed characteristic quantities:
 - Phagocytosis ratio: $p_r = \frac{N_{phag}}{N_{phag} + N_{non-phag}}$; N_{phag} = number of phagocytosed spores, $N_{non-phag}$ = number of adherent spores
 - Phagocyte adhesion ratio: $a_r = \frac{N_{adherent}}{N_{adherent} + N_{non-adherent}}$; $N_{adherent}$ = number of non-phagocytosed spores

3. Image Analysis

4. Results

5. Discussion

- We found a significant increase of the phagocytosis ratio for the virulent strain of *Lichtheimia corymbifera* in comparison with the attenuated one.
- The virulent strain might survive in alveolar macrophages and use the phagocytes as vehicles for dissemination via the blood stream causing systemic infections.

Outlook

- Quantitative answers to biological questions by an image-based systems biology approach
- High-throughput screening for different strains to perform comparative studies in an automated fashion

References

[1] Kraibooj K, Park H-R, Dahse H-M, Skerka C, Voigt K and Figge MT (2014) Virulent strain of *Lichtheimia corymbifera* shows increased phagocytosis by macrophages as revealed by automated microscopy image analysis. *Mycoses* 2014.
 [2] Mech F, Thielker A, Guller R, Brakhage AA, Figge MT. Automated image analysis of the host-pathogen interaction between phagocytes and *Aspergillus fumigatus*. *PLoS ONE* 2011; 6: e19591.

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Contact: kaswara.kraibooj@hki-jena.de

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