IDENTIFICATION OF MICROALGAE *PROTOTHECA* SPP. SPECIFIC IMMUNOGENIC PROTEINS USING PROTEOMIC ANALYSIS

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Introduction

Members of the genus *Prototheca* (*P.*) are characterized as chlorophyllless unicellular algae, which are closely related to *Chlorella* sp. Four out of six accepted species are associated with infections in vertebrates, including humans: *P. cutis*, *P. blaschkeae*, *P. wickerhamii*, and *P. zopfii* (Genotype 2). Although infections are rare, protothecosis is a severe disease with no standard treatment regime. There are three main clinical manifestations of infection: cutaneous, local and disseminated infections. The most abundant infection with *Prototheca* occurs as granulomatous mastitis in cattle. The main infectious agent appears to be *P. zopfii* genotype 2 (GT 2), whereas genotype 1

(GT 1) is classified as non-virulent^[1,2]. In addition, *P. blaschkeae* has been isolated from cattle affected by bovine mastitis. The reservoirs of the pathogenic *Prototheca* are unknown, since isolates from the environment were predominantly non-pathogenic forms. Infections with *P. zopfii* GT2 often course more severe than with the other infectious *Prototheca* species.

The study was initiated to identify the antigenic proteins of *P. zopfii* GT2 using a Western Blot and MALDI TOF MS. Cross-reactivity among the serum against *P. zopfii* GT1 and *P. blaschkeae* was also investigated.

Materials and Methods

Results and Discussion

Figure 1: 2 DE Western Blot of A: *P. zopfii* GT2 B: *P. blaschkeae* C: *P. zopfii* GT1. Immunodetection performed with hyperimmune serum obtained from a rabbit treated with *P. zopfii* GT2 each. Arrow numbers are related to the proteins in Table1.

Different *Prototheca* species or genotypes displayed a high degree of cross-reactivity (figure1). MALDI TOF MS based protein analysis of 89 spots (23 of *P. zopfii* GT2; 33 of GT1 and 35 of *P. blaschkeae*), resulted in 61 identified proteins (68 %). These were mainly involved in cellular transport, cell signaling, stress response or metabolic enzymes.

Proteomic analysis indicated three proteins that were found in every strain (table1) The translation elongation factor 1alpha was also identified in the negative control. As rabbits are herbivores, it is assumed that they were continuously exposed to plant antigens. Hence, cross-reactivity possibilities and effect due to actual immune reaction could not be distinguished. However, signal intensities from positive serum were found to be comparatively higher than that of the negative control.

At this moment, none of the identified proteins could be related to any known factor of pathogenicity or virulence factors. One of the limiting factor tend to be the detection limits of Western blot and the gel. Hence, not every Western blot signal were excised for the gel for protein identification process or an adapted metabolism to infectious life cycle serves as virulence factor itself. Western Blot analysis from the supernatant had yielded similar results in previous studies.

Spot No.	Protein full name
1	Heat shock protein 70
2	Eukaryotic translation elongation factor 1 alpha*
3	Malate dehydrogenase

Table 1. Proteins identified from *Prototheca* that were present on all three gels.

* Signal also detectable in negative control

Outlook

Serum from experimentally infected rabbits was utilized for this study. Since rabbits have never been described as a natural host of *Prototheca*, further investigations with naturally infected animals might provide clues about the mechanism of infections and the survival of *Prototheca* within host system

References:

- [1] Roesler U, Hensel A, J Clin Microbiol. (2003), 41, 3, 1181–6.
- [2] Murugaian J, Weise C, von Bergen M, Roesler U. Proteomics. 2013;13:2664–69.