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Research article

Blastomyces dermatitidis Antibody Detection with Lysate Antigens Prepared from Soil, Dog, Human and Miscellaneous Isolates of the Fungus

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Abstract

Blastomycosis is often misdiagnosed as a bacterial or viral infection; therefore, the development of improved immunodiagnostics tests has been the focus of much research in our laboratory. Much effort in our lab has focused on testing the reactivity of various lysate antigens against serum specimens from various animals. Using the ELISA technique, we tested the reactivity of 24 lysate antigens with rabbit serum. All 24 reagents were able to detect antibody in the rabbit sera. The soil antigens had the greatest reactivity with a mean absorbance value of 1.415, followed by the miscellaneous (polar bear, bat, sea lion, and cat) with 1.315, the dog with 1.279, and human with 1.039. The soil *Blastomyces dermatitidis* antigens mean absorbance values ranged from 0.934 (394) to 1.821 (85), the 6 miscellaneous lysate values ranged from 1.18 (103) to 1.595 (48938), the dog lysate values ranged from 0.966 (T-2) to 1.961 (ERC-2), and the human lysate values ranged from 0.774 (643) to 1.366 (B5927). The mean absorbance value of all the antigens was 1.262 with a range from 0.774 (ERC-2) to 1.961(643).

Keywords: *B. dermatitidis*, Blastomycosis, Antibody detection, lysate antigens, ELISA, rabbit serum

Introduction

Blastomycosis is a systemic mycosis, caused by the thermally dimorphic fungal agent *B. dermatitidis.* Infectious to humans and various animals, blastomycosis is endemic in the Southeastern and Upper Midwestern regions of the United States, including highly endemic areas of Wisconsin, Minnesota and regions of Lower Canada. *B. dermatitidis* is most prevalent in areas of moist soil and decaying matter [1, 3]. Found in a mycelial state in nature, *B. dermatitidis* spores can become airborne and inhaled into the lungs, converting to a broad-based yeast cell at 37 °C. If proper treatment is not administered, *B. dermatitidis* can disseminate to various organs, including the central nervous system, possibly leading to meningitis. Cutaneous lesions may also form as the disease progresses. Blastomycosis can prove fatal if proper treatment is not administered, particularly among the immunocompromised; therefore, early diagnosis is critical. Blastomycosis is often misdiagnosed as a viral or bacterial infection, such as tuberculosis. Although culturing and histologic diagnosis have been effective in some instances, these methods are costly and time-consuming, often delaying diagnosis until the disseminated stage of the disease [4, 10]. Therefore, the development of more efficient and reliable immunodiagnostics tests is critical, and has been the focus of much recent research in medical mycology.

Our lab has focused on the preparation and utilization of immunoassays, by testing the reactivity of lysate antigens against various serum specimens of infected and immunized animals. Promising results have been found, but more research is needed to further understand the specificity and sensitivity of the B. *dermatitidis* lysate antigens [11- 14]. Our current study used the ELISA to test the reactivity of 24 lysate antigens prepared from soil, human, canine, and miscellaneous (polar bear, bat, sea lion, and cat) isolates against serum from rabbits inoculated with *B. dermatitidis* lysate antigens.

Materials and Method

Lysate antigens

Twenty-four yeast lysate antigens were prepared from soil. dog, human and miscellaneous isolates of *B. dermatitidis* . Each of the isolates was prepared by a method similar to one that was previously used for the production of yeast lysate antigen from *Histoplasma capsulatum* [15-17] and modified in our laboratory for *B. dermatitidis* lysate antigen production [11]. The yeast phase cells were grown for 7 days at 37°C in a chemically defined medium in an incubator shaker. They were then harvested by centrifugation (700 x g; 5 min), washed with distilled water, re-suspended in distilled water and allowed to lyse for 7 days at 37°C in water with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10,000) and stored at 4°C. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Thermo-Fisher, Pierce Chemical Company, Rockford, IL) and dilutions of the antigenic reagents used in the ELISA assays were based on protein concentration.

Serum specimens

Twelve serum specimens from rabbits that were previously immunized with *B. dermatitidis* antigens were available in our laboratory and used in our comparative study.

Enzyme linked immunosorbent assay (ELISA)

The ability of each yeast lysate reagent to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA) as previously described [11-14]. Each lysate antigen was diluted (2000 ng/ml of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and then added to triplicate wells (100 ul) of a NUNC 96-well microplate (Fisher-Thermo). The plates were then incubated overnight at 4°C in a

humid chamber followed by washing three times with phosphate buffered saline containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2000 dilution; 100 ul) were added to the microplate wells in triplicate and incubated for 30 min at 37°C in a humid chamber. Following this incubation, the wells were washed as above and 100 ul of goat anti-dog IgG (H & L) or anti-rabbit IgG (H&L) peroxidase conjugate (Kirkegaard and Perry, Gaithersburg, MD, KPL) was added to each well and incubated for 30 min at 37°C. The plates were again washed as above and 100 ul of Sure Blue Reserve TMB peroxidase substrate (KPL) was added to each well and incubated for approximately 2 min at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader

Results and Discussion

Figure 1 shows that the mean absorbance of the 24 antigens ranged from 0.774 to 1.961. Although all of the antigens were able to detect antibody in the serum, the absorbance values varied. The three lysates with the greatest immunoreactivity were ERC-2 (dog, Wisconsin), 85 (soil, Georgia, ATCC 56920), and 48938 (bat, India ATCC 48938).

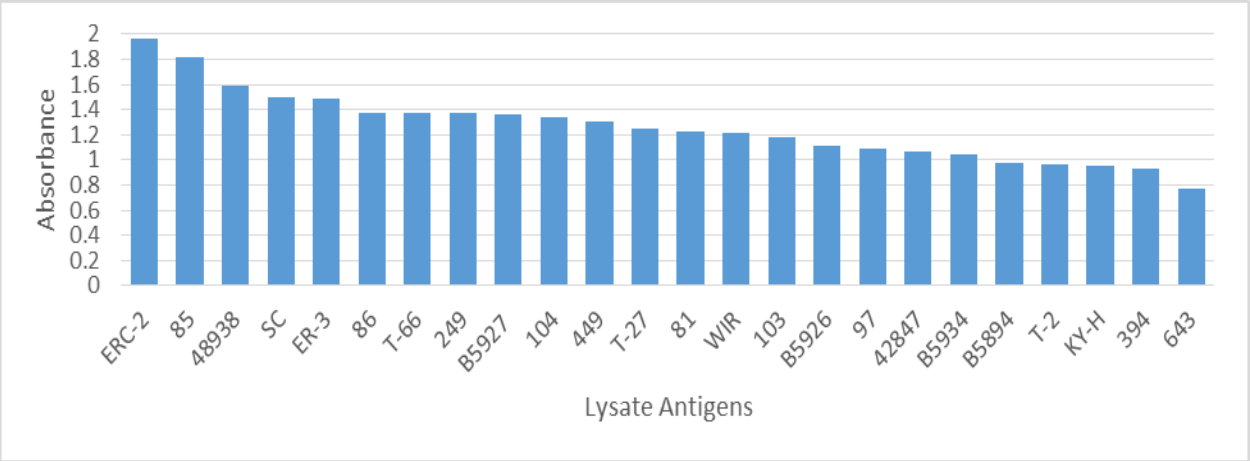

Fig. 1: Mean absorbance as determined with 24 *B. dermatitidis* lysate antigens

Figure 2 shows the mean absorbance values of the four different categories of antigens. The soil antigens had the greatest reactivity with a mean absorbance value of 1.415, followed by the miscellaneous (polar bear, bat, sea lion, and cat) with 1.315, the dog with 1.279, and human with 1.039.

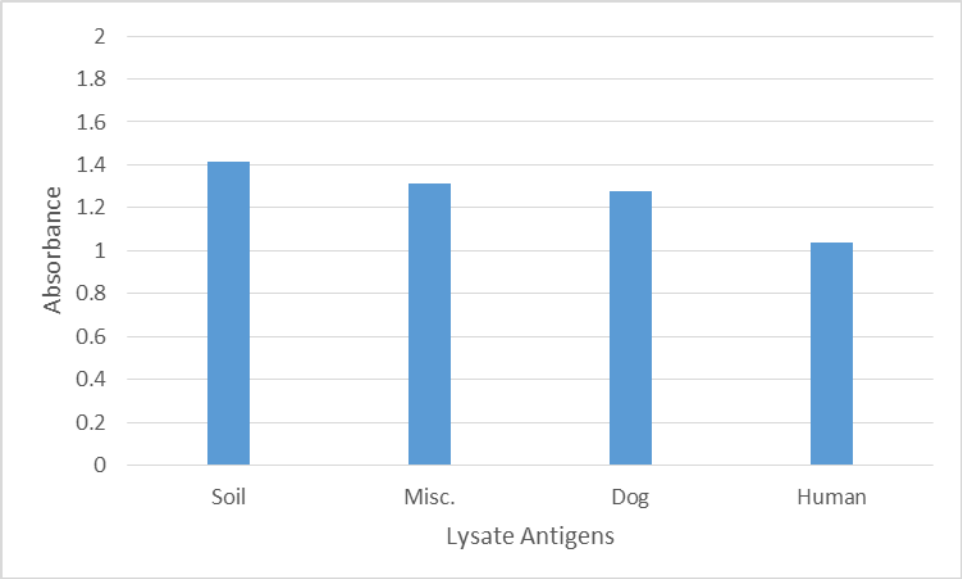

Fig. 2: Mean absorbance of each category of B. *dermatitidis* lysate antigens

Figure 3 shows the mean absorbance values obtained when the 6 lysate antigens, prepared from soil isolates, were used to detect antibody in the 12 rabbit sera. The values ranged from 0.934 (394, soil, Georgia) to 1.821 (85, soil, Georgia, ATCC 56920).

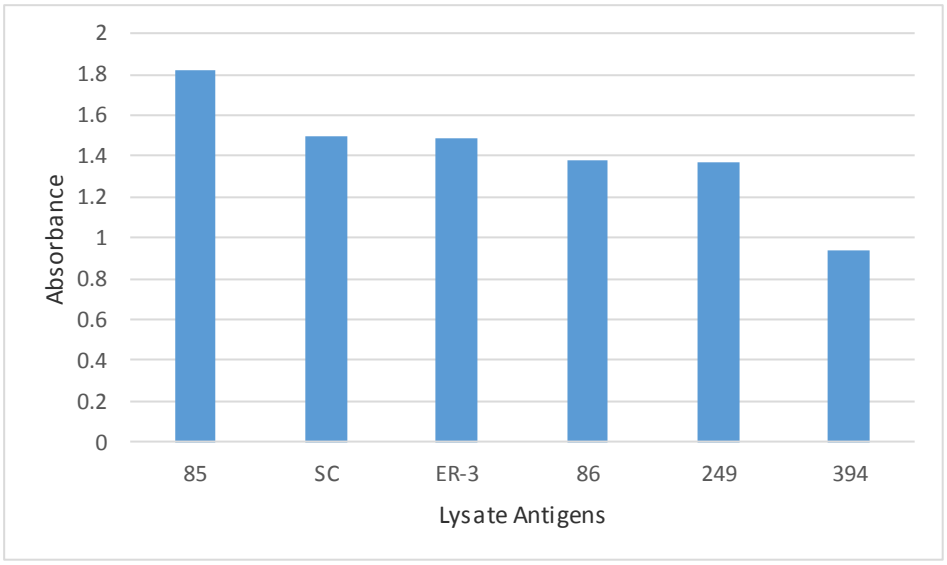

Fig. 3: Mean absorbance as determined with 6 *B. dermatitidis* lysate antigens obtained from soil isolates

Figure 4 shows the mean absorbance values obtained when the 6 lysate antigens, prepared from dog isolates, were used to detect antibody in the 12 rabbit sera. The values ranged from 0.966 (T-2 dog, Tennessee) to 1.961 (ERC-2 dog, Wisconsin).

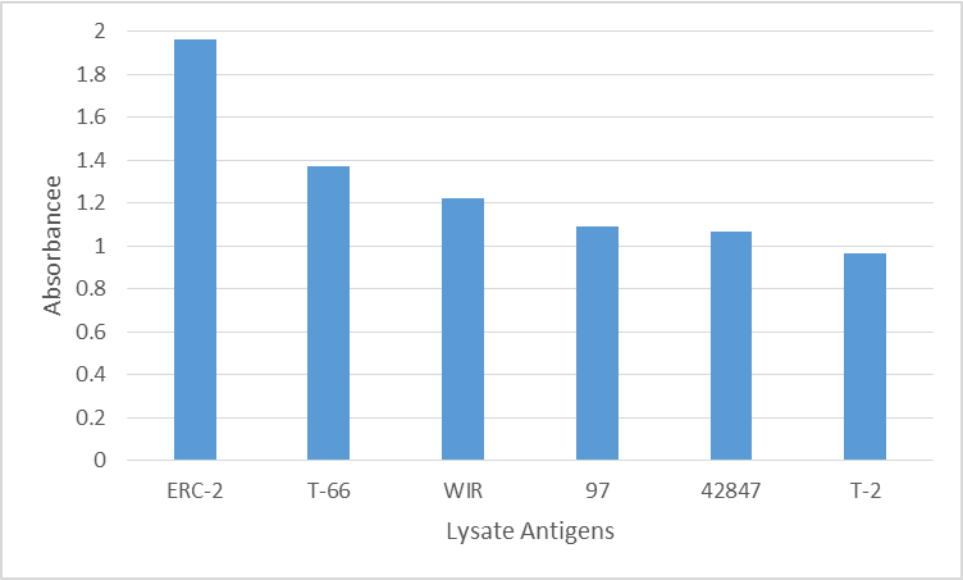

Fig. 4: Mean absorbance as determined with 6 *B. dermatitidis* lysate antigens obtained from dog isolates

Figure 5 shows the mean absorbance values obtained when the 6 lysate antigens, prepared from human *B. dermatitidis* isolates, were used to detect antibody in the 12 rabbit sera. The values ranged from 0.774 (643 Oconto Falls, Wisconsin) to 1.366 (B5927 Mountain Iron, Minnesota).

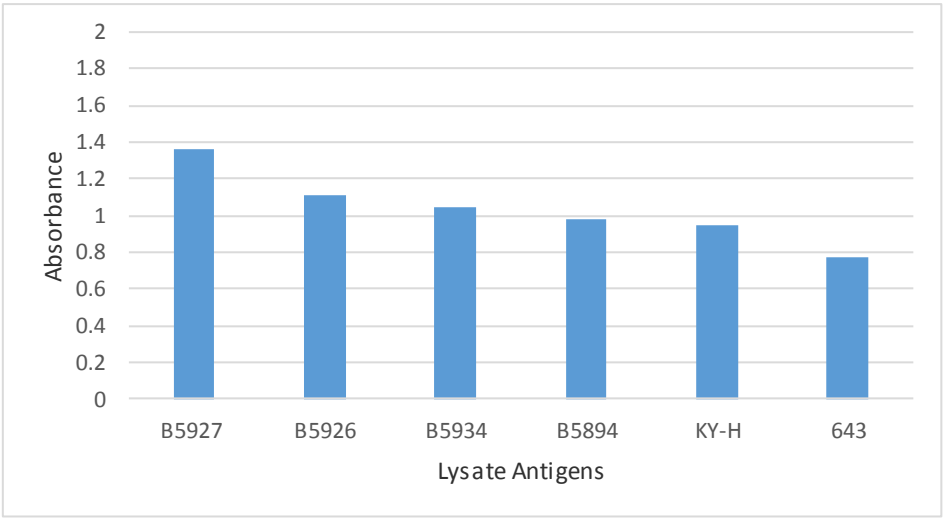

Fig. 5: Mean absorbance as determined with 6 *B. dermatitidis* lysate antigens obtained from human isolates

Figure 6 shows the mean absorbance values obtained when the 6 miscellaneous lysate antigens (48938- bat, India, 104-cat, Tennessee, 449-sealion from an Illinois zoo, T-27-polar bear from a Tennessee zoo, 81-sealion from a Tennessee zoo, and 103-cat, Tennessee) were used to detect antibody in the 12 rabbit sera. The values ranged from 1.18 (103) to 1.595 (48938).

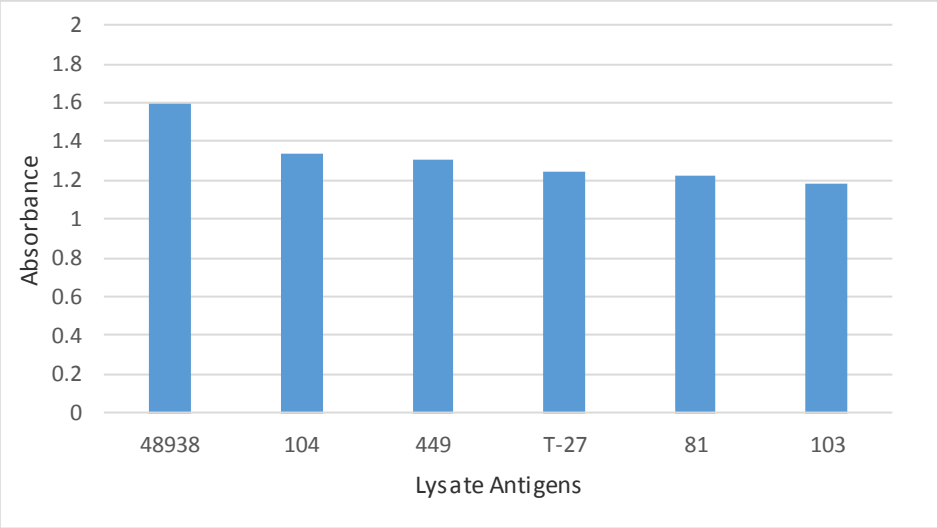

Fig. 6: Mean absorbance as determined with 6 *B. dermatitidis* lysate antigens obtained from miscellaneous isolates

Conclusion

Of the lysate antigens reacting with rabbit serum, all 24 reagents were able to detect antibody in the rabbit sera. The soil antigens had the greatest reactivity with a mean absorbance value of 1.415, followed by the miscellaneous (polar bear, bat, sea lion, and cat) with 1.315, the dog with 1.279, and human with 1.039. The soil *B. dermatitidis* isolates absorbance values ranged from 0.934 (394, Soil, Georgia) to 1.821 (85, soil, Georgia), the 6 miscellaneous lysate antigens values ranged from 1.18 (103, cat, Tennessee) to 1.595 (48938- bat, India), the dog isolates values ranged from 0.966 (T-2 dog, Tennessee) to 1.961 (ERC-2, dog, Wisconsin), and the human isolates values ranged from 0.774 (643, Oconto Falls, Wisconsin) to 1.366 (B5927, Mountain Iron, Minnesota). The mean absorbance value of all the antigens was 1.262 with a range from 0.774 (ERC-2) to 1.961(643). This study showed the efficacy of the lysate antigens as immunodiagnostic reagents in detecting antibody in serum specimens from immunized rabbits.

Acknowledgement

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