

***Blastomyces dermatitidis*: A Comparative Evaluation of Two Yeast Lysate
Antigens as Immunizing Agents**

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Abstract

Blastomycosis, caused by the dimorphic fungal agent *Blastomyces dermatitidis*, is a systemic and potentially lethal disease of humans and animals. The prevention of blastomycosis has been the subject of research investigations for many years. Comparative studies have been performed in our laboratory with respect to evaluating numerous isolates of this dimorphic fungal organism in an attempt to develop antigens that may be useful as immunogenic agents as well as *in vitro* immunodiagnostic reagents. The objective of this present study was to assay serum specimens from rabbits that were immunized with *B. dermatitidis* yeast phase lysate antigens prepared from two isolates (B5931 and B5926) in order to determine the capacity of each antigen to induce an antibody response. Serum specimens obtained on days 7, 21, 35, 49 (primary response) and on days 7 and 14 following a booster inoculation (secondary response) were assayed for antibody using seven *B. dermatitidis* antigens in the ELISA. Optimal reactivity was evidenced with the day 14 post inoculation specimens with absorbance values ranging from 0.471 to 1.273 when the B5931 lysate was used as the inducing antigen. In contrast, the B5926 antigen induced only minimal antibody with absorbance values ranging from 0.186 to 0.225. This study indicated that there are major differences in the immunizing capacity of the lysate antigens and studies are in progress to further evaluate the preparations.

Introduction

There has been a dramatic increase in systemic fungal diseases in recent years. As a

result of this increase, there has been a great deal of interest in the development of methods to stimulate protective immunity and a renewed interest in fungal vaccines (4). Currently, no vaccines are available for the potentially fatal disease blastomycosis caused by the dimorphic organism *Blastomyces dermatitidis*. Our laboratory has been conducting studies on the preparation and evaluation of various *B. dermatitidis* yeast phase lysate antigens for their ability to induce and detect antibody responses in immunized animals (1,3,8,9). This present investigation was performed to evaluate two lysate antigens (B5931 and B5926) prepared from *B. dermatitidis* isolates from a human outbreak of blastomycosis in Minnesota (2). These antigens were utilized to assess their efficacy as immunogens with regard to inducing an antibody response in rabbits immunized with each reagent.

Materials and Methods

Antigens: Yeast phase lysate antigens were prepared from two *B. dermatitidis* isolates (B5931 and B5926) by a method similar to one that was previously used for the production of antigen from *Histoplasma capsulatum* (6,7) and modified in our laboratory for *B. dermatitidis* lysate antigen production (5). The yeast phase cells were grown for seven days at 37 C in a chemically defined medium with shaking, harvested by centrifugation, followed by washing with distilled water and then allowed to lyse for seven days at 37 C in water with shaking. The reagent was 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 (Pierce Chemical Company, Rockford, IL) and dilutions of the antigenic reagents were based on protein concentration.

Immunization/serum specimens: Four New Zealand white rabbits were immunized with the two yeast lysate antigens (as above). They were inoculated intramuscularly (2ml) and subcutaneously (1ml) with an inoculum containing 500 µg/ml of protein. Serum specimens were collected on days 7, 21, 35, 49 and at days 7 and 14 following a booster immunization.

ELISA: The ability to induce antibody production by the two yeast lysate reagents was determined by the indirect enzyme-linked immunosorbent assay (ELISA; peroxidase system) using seven distinct antigens from various isolates. Each lysate antigen was diluted (1000 ng of protein/mL) in a carbonate-bicarbonate coating buffer and added to triplicate wells (100 ul) of an Immunomaxi 96-well microplate (TTP, Switzerland). The plates were incubated overnight at 4 C in a humid chamber followed by washing three times with phosphate buffered saline containing Tween 20 (PBS-T). The serum specimens (1:5000 dilution) were added to triplicate microplate wells and incubated for 30 min at 37 C in a humid chamber. The wells were again washed as above and goat anti-rabbit IgG (H & L) peroxidase conjugate (1:2000; Kirkegaard and Perry, KPL, Gaithersburg, MD) was added to each well and incubated for 30 min at 37 C. The plates were washed as above and 100 ul of peroxidase substrate (Ultra TMB; Pierce) was added to each well and incubated for approximately 2 min at room temperature. The reaction was stopped by the addition of 100 ul of 2N sulfuric acid and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader.

Results

The capability of each of the two antigenic reagents to induce antibody formation is presented in Figures 1 through 7 and summarized in Table 1. The inductive data was

determined by utilizing seven lysate antigens (as above) to detect antibody at different intervals following immunization of the rabbits. The absorbance statistics indicate that the yeast lysate antigen B5931 was the most effective at inducing an antibody response with optimal reactivity seen with the day 14 PI (post inoculation, secondary response) specimens with absorbance values ranging from 0.471 to 1.273. Conversely, the B5926 antigen induced only minimal antibody with absorbance values ranging from 0.186 to 0.225.

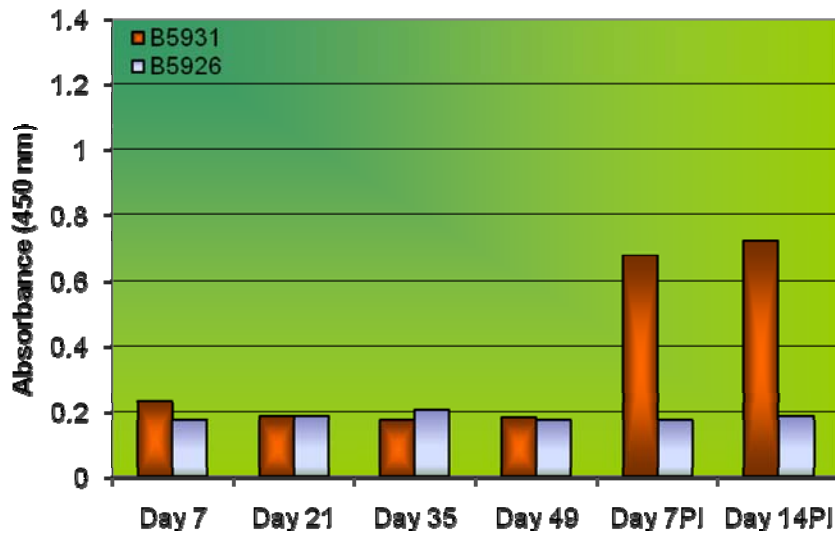


Fig. 1. Antibody induction with B5931 and B5926 lysates as detected with B5931 antigen.

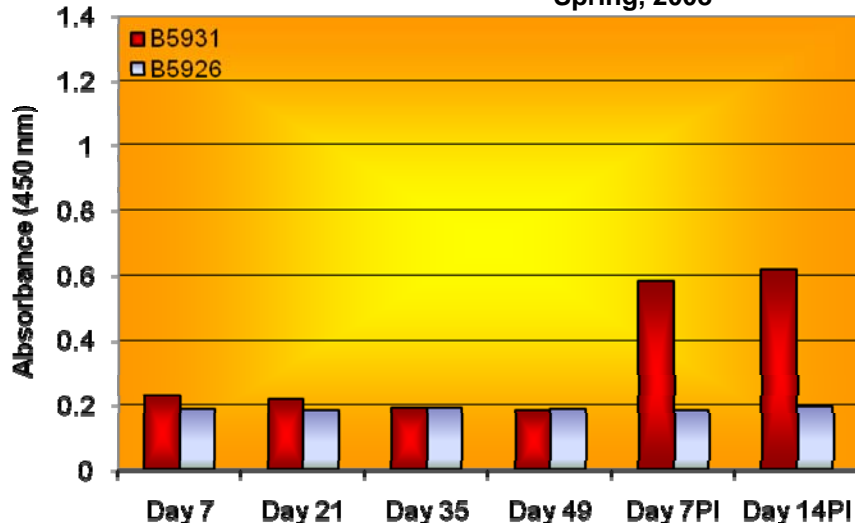


Fig. 2. Antibody induction with B5931 and B5926 lysates as detected with B5926 antigen.

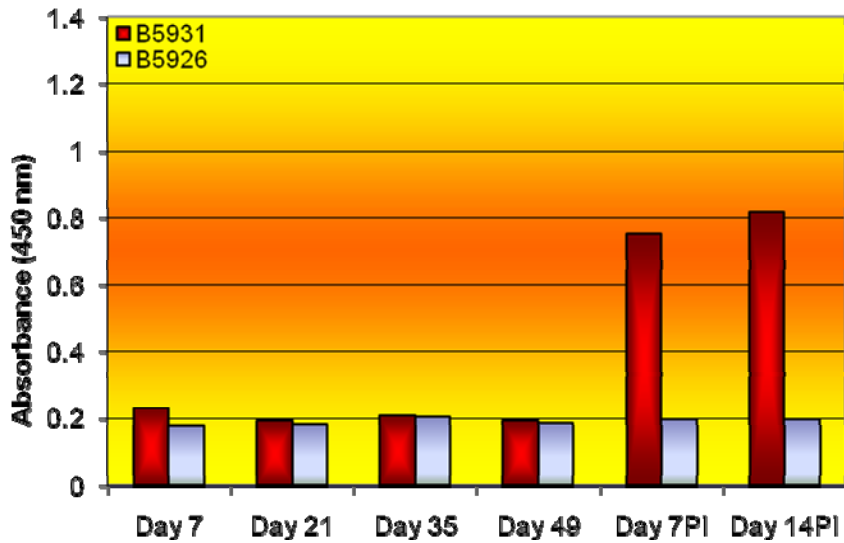


Fig. 3. Antibody induction with B5931 and B5926 lysates as detected with 48938 antigen.

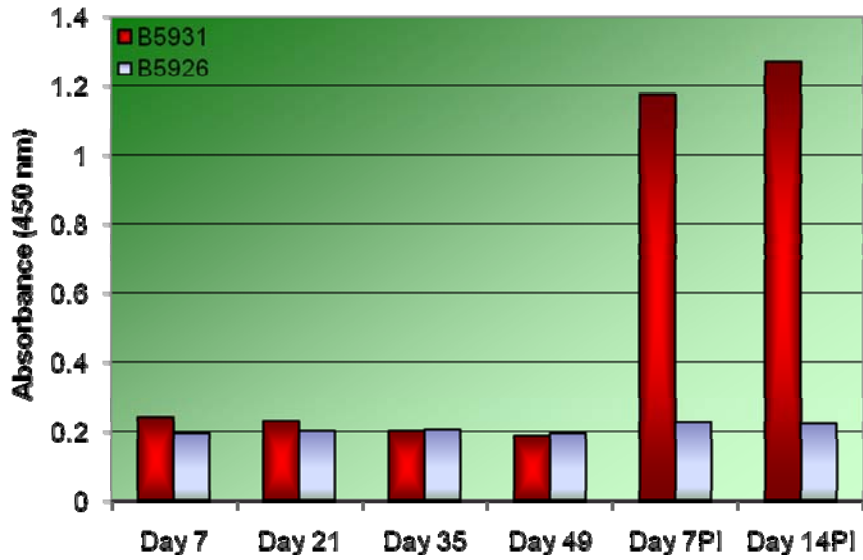


Fig. 4. Antibody induction with B5931 and B5926 lysates as detected with soil antigen.

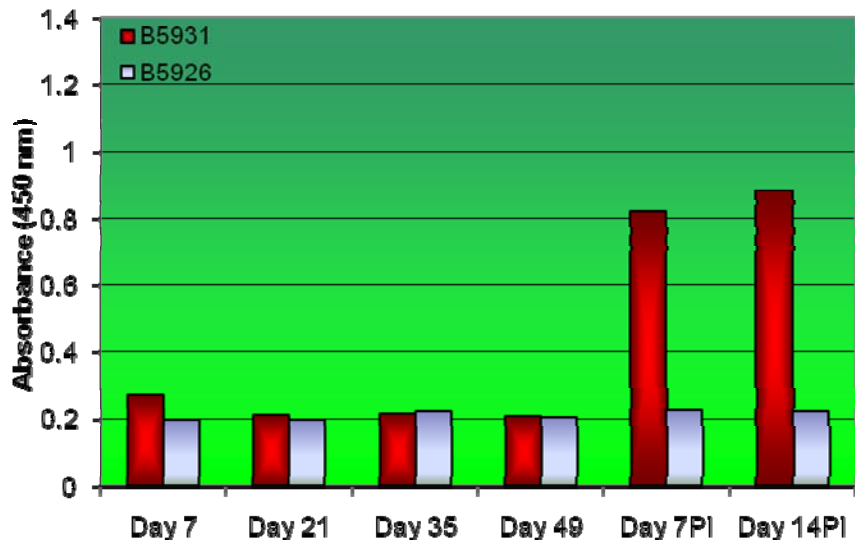


Fig. 5. Antibody induction with B5931 and B5926 lysates as detected with T-27 antigen.

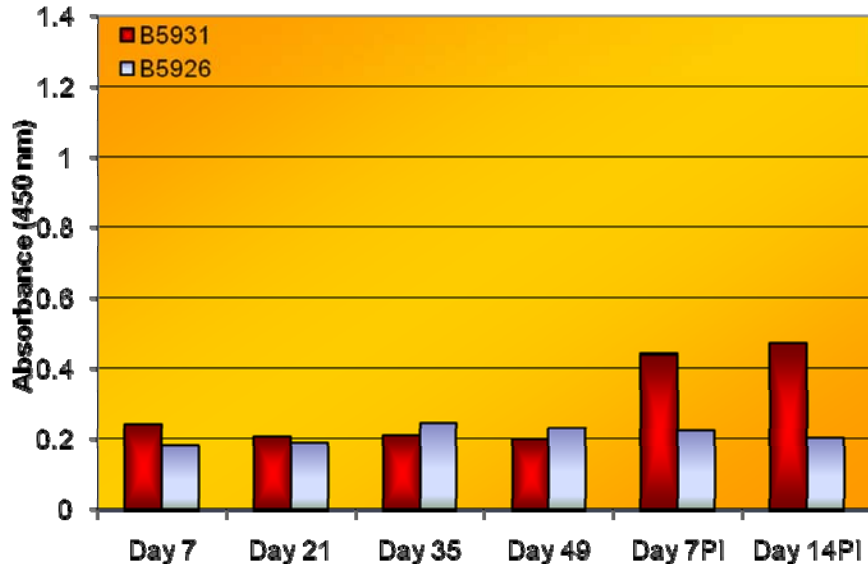


Fig. 6. Antibody induction with B5931 and B5926 lysates as detected with T-58 antigen.

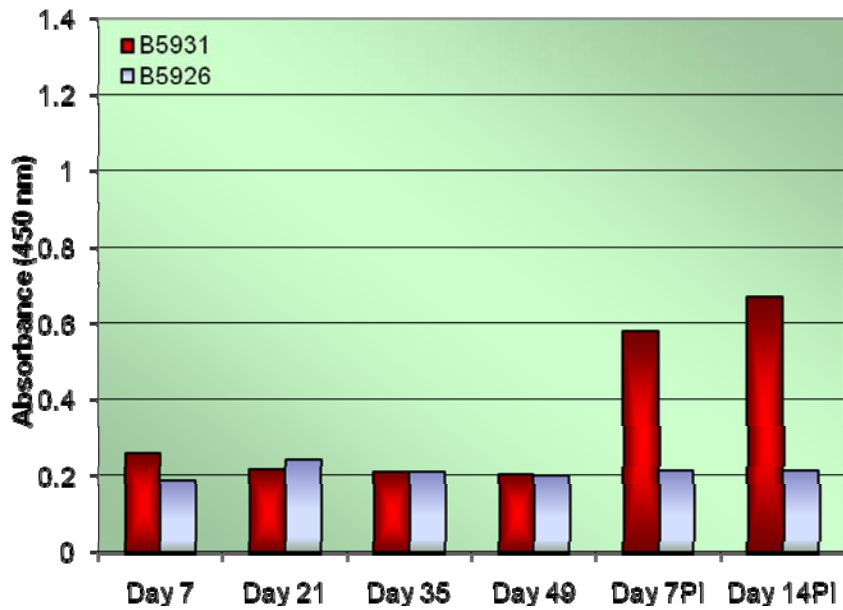


Fig. 7. Antibody induction with B5931 and B5926 lysates as detected with 449 antigen.

Table 1. Comparison of antibody induction with B5931 and B5926 antigens at days 7 and 14 PI (post inoculation, secondary response). Mean absorbance values obtained with all 7 detection reagents.

Antigen	Days	Mean Absorbance Values	Absorbance Value Range
B5931	7PI	0.719	0.433-1.177
	14PI	0.779	0.471-1.273
B5926	7PI	0.204	0.174-0.227
	14PI	0.203	0.186-0.225

Discussion

The objective of this study was to examine the antibody-inducing capability of two *B. dermatitidis* lysate antigens (B5931 and B5926) obtained from a human outbreak of blastomycosis in Minnesota. Results confirm that different strains of *B. dermatitidis* vary in their ability to induce an antibody response in the host system when the response was determined by the use of 7 different *B. dermatitidis* detection reagents.

Based on mean absorbance values measured by the ELISA, the yeast phase lysate antigen, B5931 was quite effective at inducing a high level of antibody. In contrast, the B5926 reagent showed minimal capability of inducing an antibody response in the immunized rabbits. The B5931 lysate antigen demonstrated a classic secondary immune response as the antibody production in rabbits immunized with that reagent was up to five times greater than that of the primary response. However, the B5926 strain clearly did not induce a strong primary or secondary immune response.

The results of this investigation indicate the potential for the B5931 lysate antigen to be used as an immunizing agent. Since the B5931 yeast phase lysate preparation is capable of stimulating such a strong antibody response in the host system to which it is injected into, it may possibly provide immunity for humans against the potentially fatal disease, blastomycosis, when used as an immunizing agent. Studies, including gel electrophoresis and immunoblotting, isoelectric focusing, fluorescent antibody studies on cell surface antigens, and additional research using unique *B. dermatitidis* antigenic reagents, are currently in progress.

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