

Expression of Recombinant *Aspergillus fumigatus* Antigens Using OverExpress™ C43(DE3) Cells

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Introduction

A large number of saprophytic fungi present in our environment can cause disease in humans. Among the known allergenic fungi, *Aspergillus fumigatus* is the most common species that causes invasive mycoses in immunocompromised patients. Patients at highest risk for acquiring the disease are those with a significant reduction in the number or function of granulocytes, such as leukemia patients undergoing chemotherapy or patients on long-term treatment with immunosuppressive drugs, especially cortisone (e.g., organ transplant patients). Despite antimycotic treatment, the fatality rate of invasive aspergillosis for both patient populations is approximately 58% (1). To prevent or reduce the incidence of invasive aspergillosis, we are developing a unique approach to immunization against *A. fumigatus*. Our studies are focused on developing a vaccine based on candidate *A. fumigatus* antigens that are believed to be relevant targets for providing protective immune responses against aspergillosis. Recently, we used OverExpress C43(DE3) cells to express four difficult-to-express, recombinant *A. fumigatus* antigens fused with a hydrophobic domain: AspF3-HD, AspF9-HD, Asp-hemolysin-HD, and Asp-rodA-HD. The fusion proteins will be formulated in a liposomal vaccine delivery system (2), and then tested in relevant murine models for their ability to protect against both systemic and pulmonary *A. fumigatus* challenge.

Materials and Methods

Recombinant DNA for each of the five *A. fumigatus* antigen genes was synthesized, ligated into the pET28a vector, and transformed into OverExpress C43 (DE3) *E. coli* cells. Cells were plated onto LB-agar* plates containing 30 µg/ml kanamycin. Positive transformants were identified by restriction enzyme analysis and DNA sequencing. One clone for each *A. fumigatus* antigen was selected for protein expression and purification.

Each positive clone was grown in 0.5 L of TB medium with 30 µg/ml kanamycin. Protein expression was induced with 0.75 mM IPTG, when the culture reached an OD₆₀₀ of 0.5, and then incubated for an additional 6 hours. The cells were pelleted at 6,000 x g for 30 min, resuspended in an 8 M urea buffer, and lysed by microfluidization at 15,000 psi. Following centrifugation at 30,000 x g for 1 h, clarified lysate was loaded onto an 8-ml Ni-NTA agarose column, washed with 5 volumes of 8 M urea buffer, 5 volumes of 0.3% sodium deoxycholate buffer, and 5 volumes of 50 mM sodium phosphate buffer. The 6xHis-tagged proteins were eluted with 5 volumes of 250 mM imidazole elution buffer. Column fractions were analyzed by SDS-PAGE. Protein concentrations were estimated using both UV absorbance at 280 nm and the bicinchoninic acid assay. Endotoxin levels for each protein preparation were estimated using the Limulus amoebocyte lysate assay.

Results and Discussion

Four recombinant *A. fumigatus* antigens were successfully purified after expression in OverExpress C43 (DE3) cells. Each protein eluted from the Ni-NTA agarose column with an elution buffer containing 250 mM imidazole. SDS-PAGE (Figure 1) showed that each protein was expressed and purified. After purification, the following protein concentrations were estimated (per liter of fermentation): 0.9037 mg/L for AspF3-HD, 3.6437 mg/L for AspF9-HD, 1.3006 mg/L for Asp-hemolysin-HD, and 1.8105 mg/L for Asp-rodA-HD.

Conclusions

Using OverExpress C43(DE3) *E. coli* cells, we successfully expressed four recombinant *A. fumigatus* antigens: AspF3-HD, AspF9-HD, Asp-hemolysin-HD, and Asp-rodA-HD. Because of the high efficiency of expression for these hydrophobic fusion proteins, which have proved difficult to express in other cells, we will now be able to assess the protective immune response elicited by each of the five *A. fumigatus* antigens.

References

1. Lin, S. J. et al. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. 32, 358-366 (2001).
2. Ernst, W. A. et al. Protection against H1, H5, H6 and H9 influenza A infection with liposomal matrix 2 epitope vaccines. Vaccine. 24, 5158-5168 (2006).

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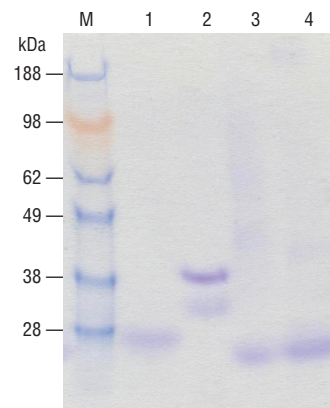


Figure 1. High protein yield. SDS-PAGE on a 4-20% Tris-HEPES gel shows high protein yield for each of the four *A. fumigatus* antigens. Lane M, molecular weight marker; Lanes 1-4, purified protein samples: (1)AspF3-HD; (2)AspF9-HD; (3)Asp-hemolysin-HD; (4)Asp-rodA-HD. 20 µg of sample was loaded per well.