

FIG S1. Expression of Cfp4 fragments fused to GST in *E. coli*. Fragments of Cfp4 were fused in frame to the C-terminus of GST using vector pGEX2 and transformed into *E. coli* strain TG1. Fusion protein expression was induced for 3 hours by addition of 0.5 μ M isopropyl- β -D-thiogalactopyranoside (IPTG). Cellular lysates were prepared from bacterial cells by boiling of cells in 1X Laemmli sample buffer. Lysate proteins were separated by SDS-polyacrylamide gel electrophoresis using 13% acrylamide. Proteins were visualized by Coomassie staining. Asterisks indicate unique protein bands representing the GST:Cfp4 fusions encoded on plasmids pCR617 (amino acids 70-113), pCR618 (amino acids 98-202), and pCR619 (amino acids 87-169).



FIG S2. Anti-Cfp4 immunoblot of wild-type *Histoplasma* yeast culture filtrates. Culture filtrate proteins (with and without prior deglycosylation with PNGaseF) were separated by electrophoresis through 12% acrylamide and transferred to nitrocellullulose. Cfp4 protein was visualized by immunooblot with 2D20 monocolonal antibody to Cfp4

FIG S2





FIG S3. Classification of North American clinical *Histoplasma* **isolates by sequence polymorphisms.** Genomic DNA was prepared from clinical isolates Hc06, Hc20, Hc01, Hc10, Hc16, Hc22, Hc27 obtained from the OSU Medical Center. Strains were assigned to North American type I (NAm1) or type 2 (NAm2) phylogenetic groups by lineage-specific nucleotide sequence polymorphisms following PCR-based amplification of the *YPS3* and *SOD3* loci. (A) PCR size polymorphisms in the *YPS3* gene were used to assign isolates to NAm1 (630 bp) or NAm2 (339 bp). Panama classification also shown using the Panama strain G186A. (B) PCR-RFLP polymorphisms in the *SOD3* gene were based on lineage-specific XhoI restriction sites in a 772-775 bp *SOD3* amplicon. XhoI-digested PCR amplicons were predicted to generate the following fragments: NAm1 (1 XhoI site yielding 358 bp and 417 bp fragments), NAm2 (1 XhoI site yielding 62 bp and 712 bp fragments), and Panama (no XhoI site yielding a 772 bp product).