The *Aspergillus fumigatus* morphogenesis-related kinase, CotA, orchestrates hyphal growth in response to carbon source quality

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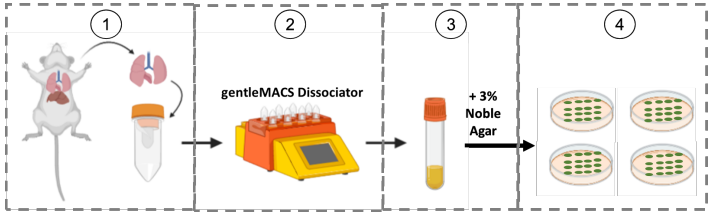
Introduction

Fungal pathogens' ability to grow in stressful environments, like the infected host, is the result of an intricated network of molecular processes that result in efficient nutritional plasticity. We recently generated a protein kinase disruption library in the opportunistic fungus *Aspergillus fumigatus*. The objective of this study was to identify and characterize protein kinases required for pathogenic growth in this pathogen.

Results



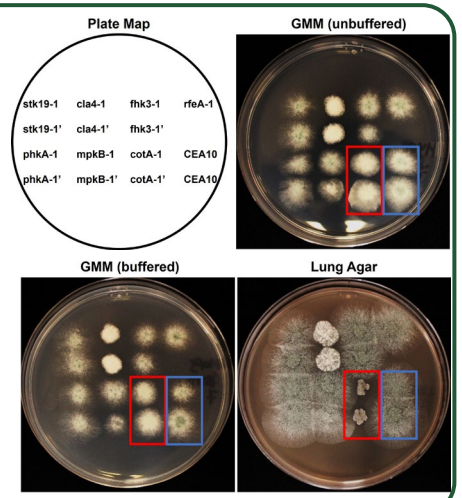
Generation of a culture medium mimicking the host environment.

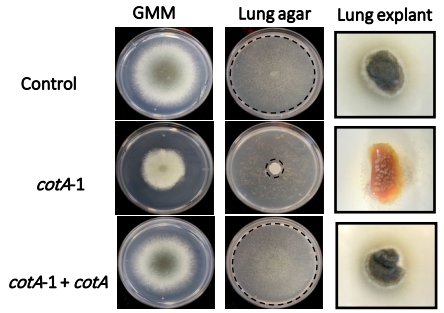
Lungs from healthy mice were aseptically removed and homogenized in 0.165M MOPS, pH 7 containing 2x gentamycin and chloramphenicol. The lung homogenates were then mixed with equal amounts of 3% Noble agar and poured over Petri dishes.

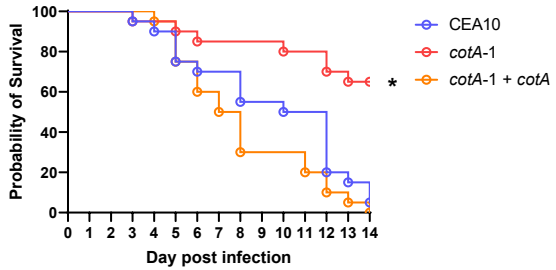
Screening of a kinase disruption library using Lung Agar.

Five microliters containing 10^4 conidia were inoculated in lung agar plates as well as in glucose minimal media (GMM) buffered with 0.165M MOPS or regular GMM supplemented with antibiotics, as growth controls. Twenty different strains, containing two biological replicates for each mutant, were analyzed in each petri dish and the parental strain, CEA10, was included in every plate (blue rectangles). The plates were incubated at 37°C and the growth was analyzed every 24 hours for a period of 72 hours.

The red rectangles highlight the *cotA*-1 mutant, showing a strong hyphal development defect in lung agar, compared to GMM and compared to CEA10.



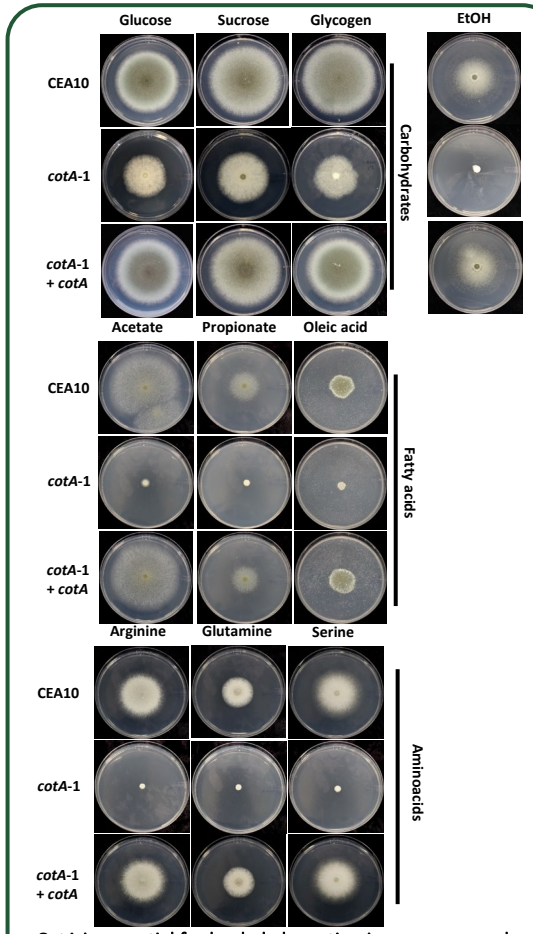




The *cotA*-1 mutant displays reduced growth in lung agar, lung explants and reduced virulence in a mouse model of IA.

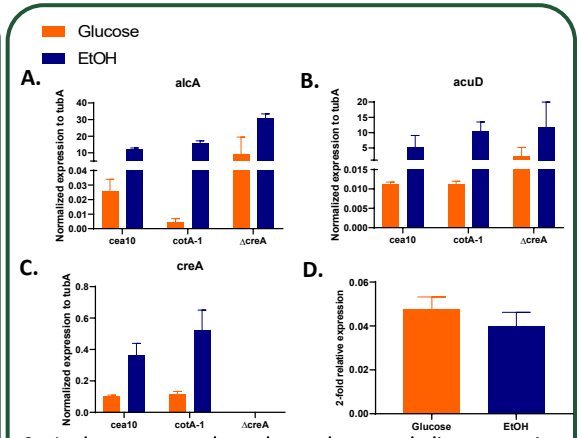
Ten thousand conidia were spotted onto GMM, lung agar plates or lung explants and colony diameters were measured every 24h during 96h. Dotted line delimites perimeter of the colony.

For the mouse model of invasive aspergillosis, CD-1 female mice were immunosuppressed with cyclophosphamide and triamcinolone acetonide, and inoculated with 10^5 conidia. Survival was monitored for 14 days and plotted by Kaplan Meier curve, and analyzed using log rank test in GraphPad Prism v 8.2.1.



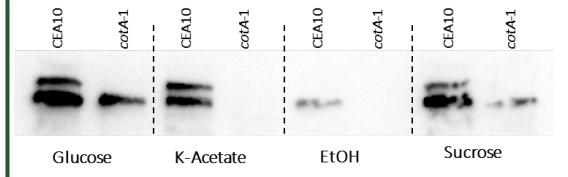
CotA is essential for hyphal elongation in non-sugar carbon sources.

Ten thousand conidia were inoculated in the center of minimal media agar containing different carbon sources. The plates were incubated at 37°C for 96 hours.



CotA does not regulate the carbon catabolite repression system, and *cotA* gene expression is not regulated by it.

The different strains were grown in glucose minimal media (GMM) for 16 hours at 37°C. After this time, the media was replaced by EtOH minimal media or by GMM and the cultures were incubated for an additional 2 hours. Then, RNA was extracted and the expression of *creA*-controlled genes (*alcA* [A] and *acuD* [B]), as well as *creA* [C] expression levels were determined by RT-qPCR. *cotA* expression [D] was also assessed in the Δ*creA* strain. The Δ*creA* mutant was grown as explained in A_C. Then, RNA was extracted and the *cotA* mRNA levels were determined by RT-qPCR.



The CotA protein exerts isoform-specific control over hyphal growth on alternative carbon sources.

Three miligrams of total protein were immunoprecipitated using GFP-trap magnetic beads. The proteins were then separated on a 12% acrylamide gel and transferred to a PVDF membrane. CotA was detected using a novel antibody raised against the kinase domain of *A. fumigatus* CotA.

Conclusions

The *A. fumigatus cotA* gene encodes a conserved morphogenesis-related kinase that is produced as two protein isoforms, long and short. The presence of the long isoform is required for virulence in a manner dependent of host-relevant carbon source quality.