Characterizing the *Aspergillus nidulans* Kinase Deletion Library for Increased Septation in Response to Cell Wall Stress

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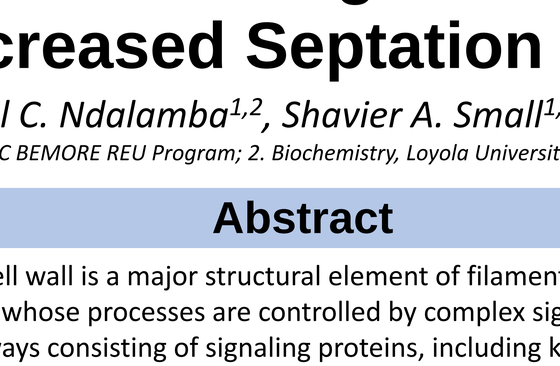
Abstract

The cell wall is a major structural element of filamentous fungi, whose processes are controlled by complex signaling pathways consisting of signaling proteins, including kinases. To determine the role of *Aspergillus nidulans* protein kinases in the fungal response to cell wall stress, we grew multiple strains from a *A. nidulans* kinase deletion library, each of which has a single kinase deletion. We hypothesize that some of these protein kinases are involved in the cell-wall-integrity signaling (CWIS) pathway and/or regulation of septation. To test this hypothesis, we grew strains over 16-hour experiments under two conditions: treating one with micafungin, a cell wall perturbant, at the 12-hour mark and not disturbing the other. After 16 hours, we imaged the fungi and determined number of septa and the projected area in each of approximately 30 mycelia. Through observing the extent of growth and septation, we can draw conclusions about the role each kinase performs within the CWIS pathway.



Chelius et al., 2020, *Mol Cell Proteomics 19(8)*, 1310–1329

Figure 1. Previous research from our lab implies micafungin induces a stress response through the CWIS pathway causing an increase in septation.

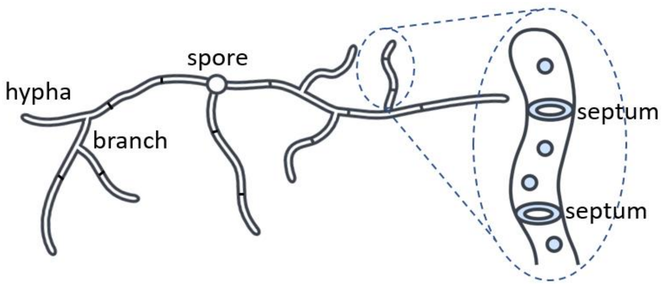


Figure 2. Schematic of fungal mycelium. Hyphae grow from spore, forming branches and septa as they elongate. Septa divide a hypha into cellular compartments. The central septal pore can close when the cell wall encounters stress, preserving the hypha despite damage to a septal compartment.

Materials and Methods

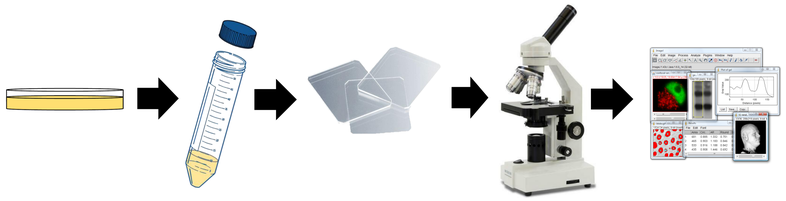
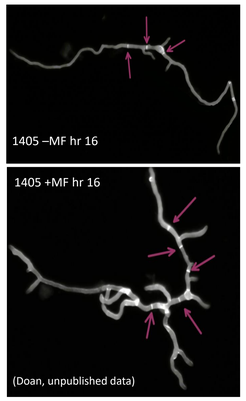


Figure 3. Schematic representation of spore growth, harvesting, imaging, and analysis process.



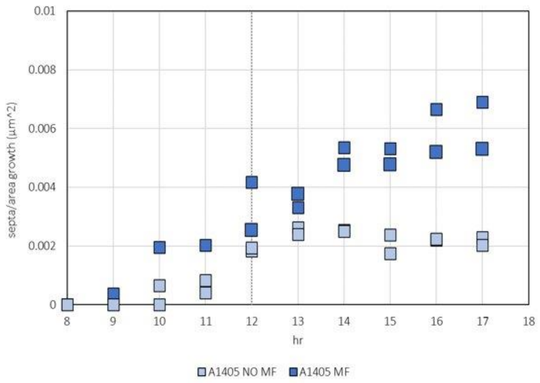


Figure 4. Photos show **c**ontrol strain (*A. nidulans* 1405) exhibits increased septation (red arrows) under cell-wall stress from micafungin. Graph shows septation time-course, both with (dark blue squares) and without (light blue squares) micafungin. When micafungin is present, A1405 exhibits increased septation per hyphal area.

Results

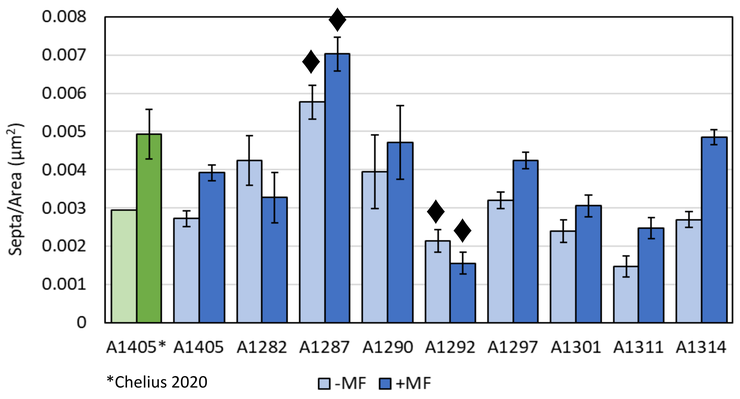


Figure 5. shows the average septa per area for 10 different strains, including control strain A1405 (i.e., no kinase deletion). Note that A1405* is previously published data. The light-colored bars indicate the condition with no micafungin and the dark-colored bars indicate the condition with micafungin. A1287 shows a statistically significant increase (♦) in septation from the control, where A1292 shows a statistically significant decrease (♦).

Discussion

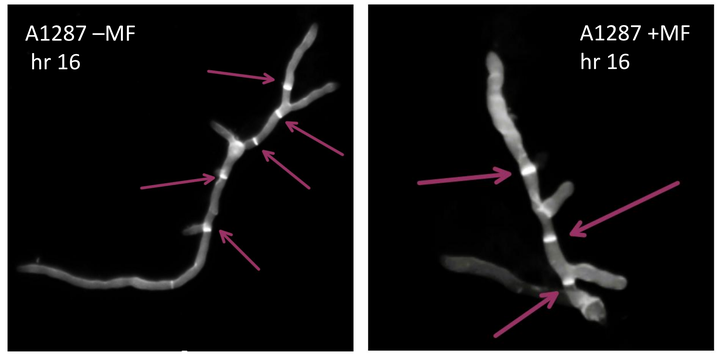


Figure 6. Strain A1287 (i.e., deletion of AN0699; Δ*cak1*) shows increased levels of septation (compared to control strain A1405) both with and without micafungin. *A.nidulans cak1* is a homologe to *S. cerevisiae CDC28*, whose gene product is a master regulator of mitotic and meiotic cell cycles. We hypothesize Cak1 kinase mediates suppression of septation, so that in its absence this strain has higher levels of septation.

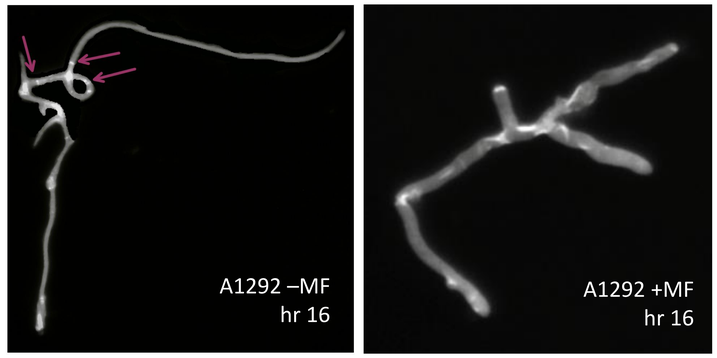


Figure 7. Strain A1292 (i.e., deletion of AN10082; Δ*srpkF*) shows decreased levels of septation (compared to control strain A1405) both with and without micafungin. *A.nidulans srpkF* is a homologe to *S. cerevisiae SKY1*, whose gene product is a component of stress granules and regulates granule disassembly during recovery from stress. From our observation of reduced septation, we hypothesize that SrpkF kinase is involved in the induction of septation.

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