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## Abstract

A complete understanding of the underlying growth and development of filamentous fungi remains largely unknown. Current research interests concerning filamentous fungi such as *Aspergillus nidulans* have to do with their characteristic polarized growth. It is thought that endocytosis is required for the continuation of such growth. The proteins AmpA and AmpB are believed to play a role in the final step(s) of endocytosis. In this study, strains were examined in which *ampA* or *ampB* were down regulated. Phenotypic variables that were considered included: radial growth rate, conidiation, and germination. The down regulation of *ampB* resulted in reduced germination and conidiation compared to wild type. It is worth noting that AmpA and AmpB might have overlapping function. This hypothesis could be tested by crossing the *ampA* and *ampB* strains to each other to produce the double mutant. When *ampB* was repressed, this resulted in abnormal development of the fungus.

## Introduction and Objectives

Polarized growth has been a perplexing issue in understanding the overall structure and functions within filamentous fungi. Over the past decade, it has become generally accepted that the maintenance of this polar growth is a direct result of a precise balance between exo- and endocytosis (see Shaw et al. 2011). The investigated genes, *ampA* and *ampB* are thought to play an integral role as part of the scission machinery of filamentous fungi during endocytosis (see Youn et al. 2010; see Peñalva 2010). The *niiA* promoter was inserted in front of the *ampA* and *ampB*. This promoter represses expression in the presence of ammonia. Objectives included:

- Determine the role of the Amphiphysins (AmpA/AmpB) as they relate to morphology and physiology
- Compare growth and phenotypic expression between wild type, *ampA*, and *ampB*
- Hypothesis: When the expression of the proteins AmpA and AmpB are repressed abnormal growth and development will occur in the fungus

## Methods

### Radial Growth

- Wild type, *ampA*, and *ampB* were plated three times (each) on Minimal Media (MM-) with Nitrate (KNO<sub>3</sub>), Ammonia (NH<sub>4</sub>Cl), Nitrate w/ 0.4M Salt, Ammonia w/ 0.4M Salt, Nitrate w/ 0.8M Salt, or Ammonia w/ 0.8M Salt (see Higuchi et al. 2009)
- Measured hyphal extension each day for 4 days

### Conidiation

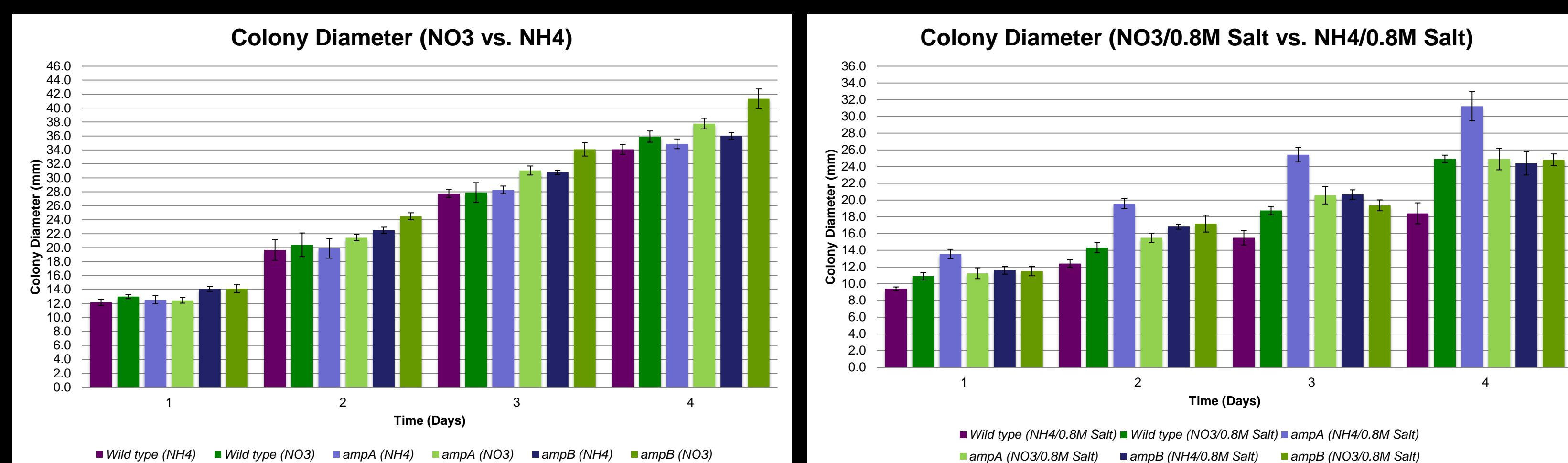
- Plated wild type, *ampA*, and *ampB* on nitrate- and ammonia-based media
- Used standard concentration of 1x10<sup>5</sup> spores/mL
- 0.79 cm<sup>2</sup> sections of culture were collected after 5 days incubation at 28 degrees C
- These sections were then homogenized in 1 mL ddH<sub>2</sub>O and spore concentrations were measured using a hemocytometer

### Germination

- Incubated conidia in liquid ammonia- or nitrate-based media
- 4000 conidia were used for each of three replicates
- Germination was assessed after 7 hours (n=450)
- Percentage germinated was then calculated

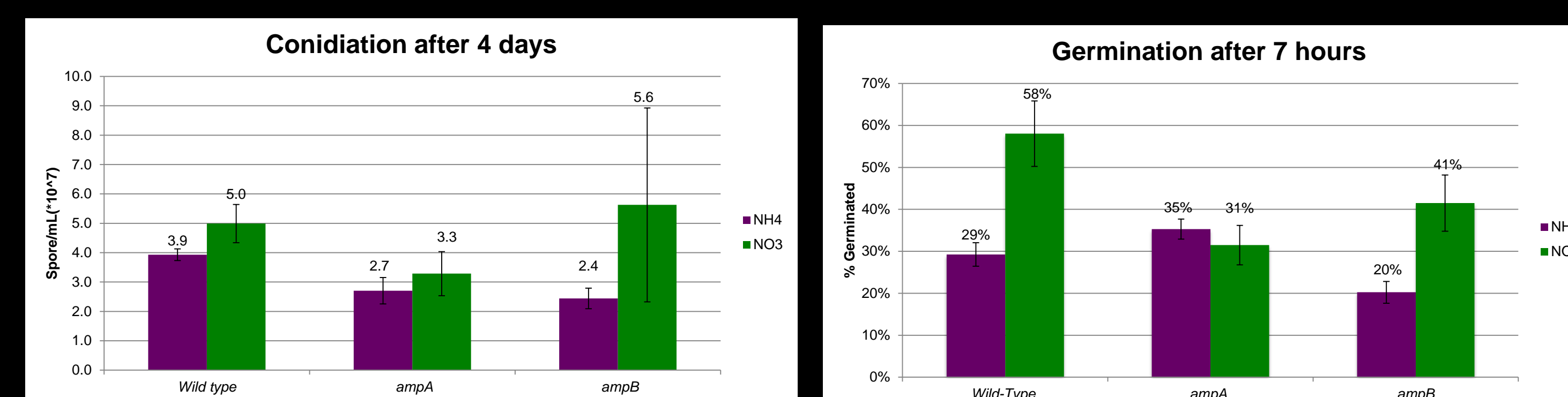
## Results

The colony growth rates of the mutants were compared to wild type on inductive and repressive media. No significant difference was noted for the mutants when compared to wild type.

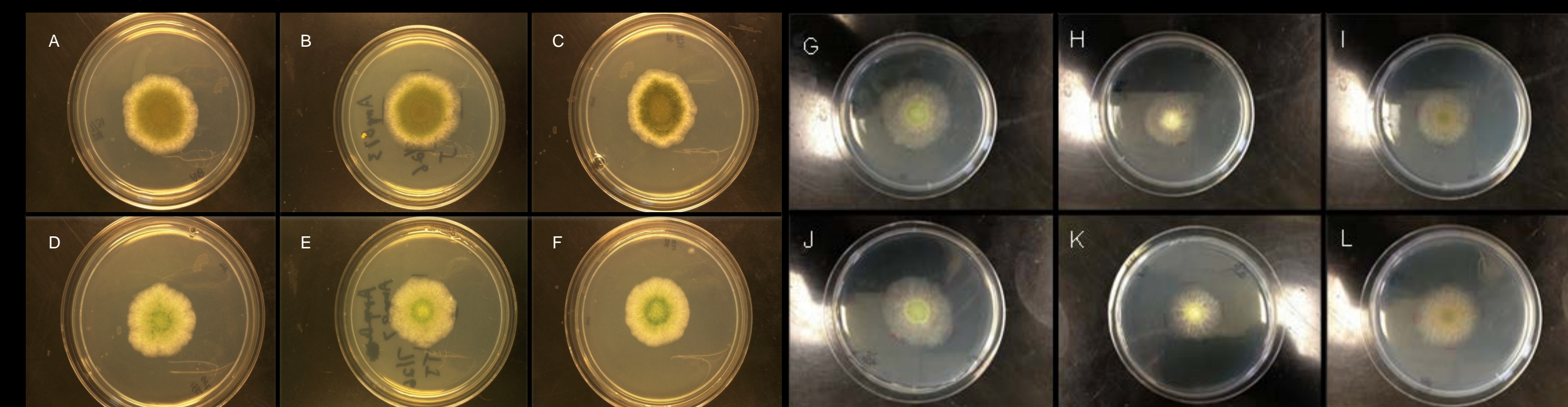


Figure(s) 1&2. Charts of average colony diameter each day for 4 days. There was no significant difference in growth rate comparing wild type to the mutant strains.

Fewer conidia were produced by *ampA* (1.2x10<sup>7</sup> conidia/mL; p-value: 0.069) and *ampB* (1.5x10<sup>7</sup> conidia/mL; p-value: 0.019) on ammonia-based medium compared to wild type. Conidia from *ampB* (p-value: 0.023) cultured on ammonia germinated approximately 9% less than wild type.



Figure(s) 3&4. Data for conidiation after 4 days and germination after 7 hours. Wild type produced more conidia than *ampA* and *ampB* on ammonia-based media. Wild type germinated better than *ampB* on ammonia, but not *ampA*. Germination was defined as the emergence of a germ tube.



Figure(s) 5&6. Visualization of radial growth after 4 days. Samples grown on ammonia were relatively smaller than those grown on nitrate [D,E,F vs. A,B,C], but no difference was observed between the mutants and wild type grown on ammonia [D,E,F]. Samples grown with 0.8M salt [H,I,K,L] were smaller than those grown on 0.4M salt [G,J]. No clear distinction was noted between the strains. In order from left to right: *ampA*, *ampB*, wild type [A-F]; ammonia 0.4M salt, ammonia 0.8M salt, nitrate 0.8M salt [G-L]. Top to bottom: nitrate, ammonia [A-F]; *ampA*, *ampB* [G-L].

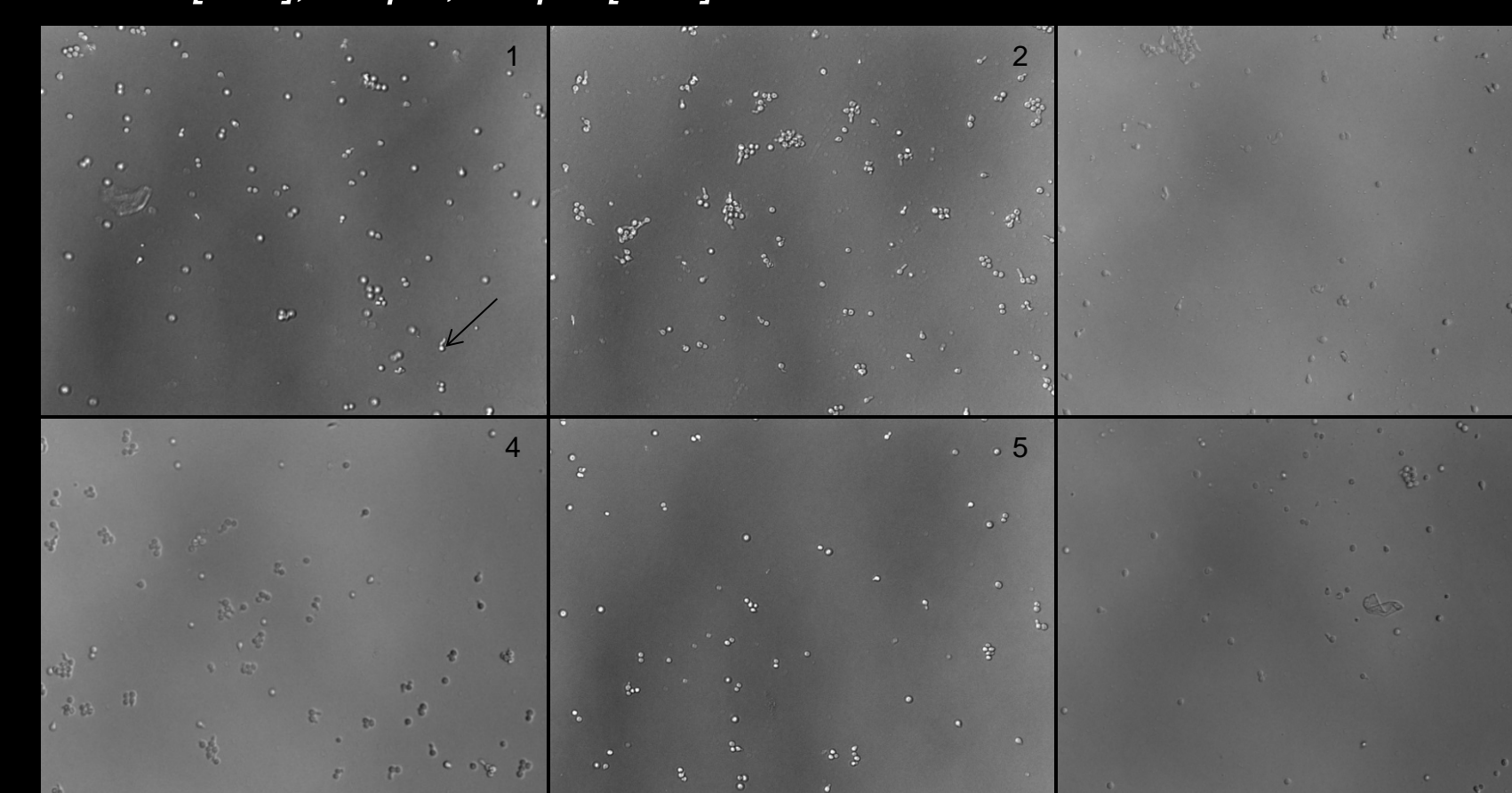


Figure 7. Germination after 7 hours in nitrate (top) and ammonia (bottom) liquid media. No difference was observed in *ampA* [(1);(4)]. Wild type germinated better than *ampB* in ammonia [(6)- w.t. ammonia; (5)- *ampB* ammonia]. Arrow indicates a germinated conidium. In order from left to right: *ampA*, *ampB*, wild type.

## Conclusion

- Abnormal radial growth was not observed when either *ampA* or *ampB* were repressed
- Conidiation and germination were both significantly affected by the down regulation of *ampB*; the down regulation of *ampA* reduced conidiation
- Further evidence is required to fully understand the role of the proteins Amphiphysin A&B
- AmpA and AmpB might be functionally redundant, producing normal hyphal growth and development even when one is repressed

## References

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