# Evidence-Based Genome Annotation for Degradative Enzymes in fungal pathogen, *Cryptococcus neoformans*

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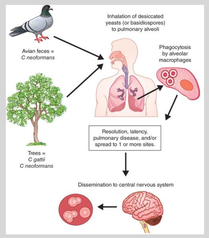
## Backround




Brooks, et al, 2016

*Cryptococcus neoformans (Cn)* is a fungal pathogen that most people have been exposed to, but in immunocompromised patients, the infection can be deadly. First contact occurs when spores that are found in soil are inhaled and the pathogen spreads from the lungs to the central nervous system leading to meningitis (McClelland et al. 2007).

- Virulence factors are phenotypic traits that aid in the ability of a pathogen such as Cn to infect the human host. This project will be focus on the production of degradative enzymes, which are specific virulence factors: urease and phospholipase B.
- Urease facilitates blood to brain transmission (Ma and May 2009). Phospholipase is necessary for the distribution of Cn in the lungs but not necessarily the survival (McCllelland et al. 2007). Phospholipase B reduce the immune response of the host (McClelland et al. 2007).
- These two degradative enzymes are each encoding by separate genes which also have proteins that directly and indirectly regulate their production. However, many aspects of this pathogen's genome and functional annotation are yet unknown.

| Degradative Enzyme | Gene | Gene Location |
|---|---|---|
| Phospholipase B | CNAG 06085 | Chromosome 12 at 260467-263663 |
| Urease (Ure1) | CNAG 05540 | Chromosome 14 at 596591-600484 |

| Associated Proteins | Gene | Gene Location |
|---|---|---|
| Urease Accessory Protein (Ure G) | CNAG 00678 | Chromosome 1 at 1761229-1763892 |
| Urease Accessory Protein | CNAG 01166 | Chromosome 5 at 1026129-1027326 |

## Purpose

The purpose of this research is to observe and annotate the degradative enzyme genes of the *Cryptococcus neoformans* genome.

## Methods

Used Apollo to compare PASA version of gene to the gene in the strains. Look at the evidence to see if genes align. The exons that are not aligned will be annotated.

## Results

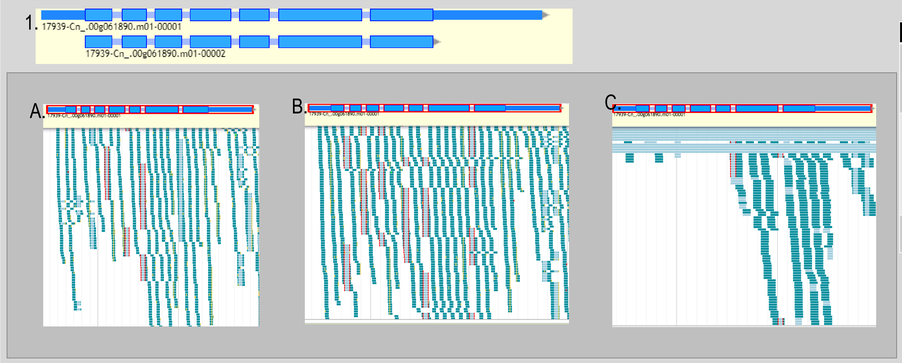



**Table 1: Phospholipase B Gene**

| Figure | Strains | Exon Problems |
|---|---|---|
| *A* | B15 replicate 01, B40 replicate 01, B30 replicates 01 and 02, B43, B45, and B58 | Exons 1, 2, 5, 6: not defined Exons 3 and 4: missing |
| *B* | B15 replicate 02 and replicate 03, B18, B40 replicate 02 and replicate 03, H99S_RM1-3 H99S_Sc100_RM1-3 | Exons 1 and 7: longer |
| *C* | H99S_Sc100_T_RM1-3 | Exons 1, 4, 5, 6, 7; missing |

**Figure 1: Shows the revised PASA version and original genome of Phospholipase B**

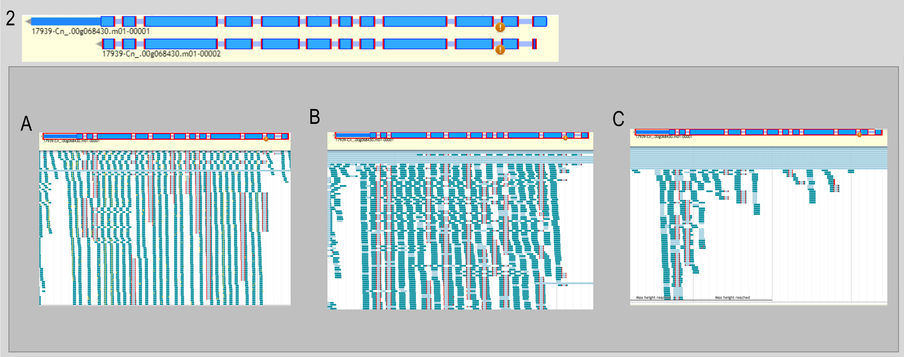



**Table 2: Ure 1**

| Figure | Strains | Exon Problems |
|---|---|---|
| *A* | B15, B18, B40, B30, B43, B45, B58 | Exon 1: longer Exons 11 and 12: missing |
| *B* | H99S_RM1-3 H99S_Sc100 _RM1-3 | Exons 1: longer Exons 10,11, and 12: missing |
| *C* | H99S_Sc100 _T_RM1-3 | Exon 1: longer Exons 3, 4, 5, 7, 8, and 9: not defined Exons 10, 11, and 12: missing |

**Figure 2: Shows the revised PASA version and original genome of Urease (Ure1)**

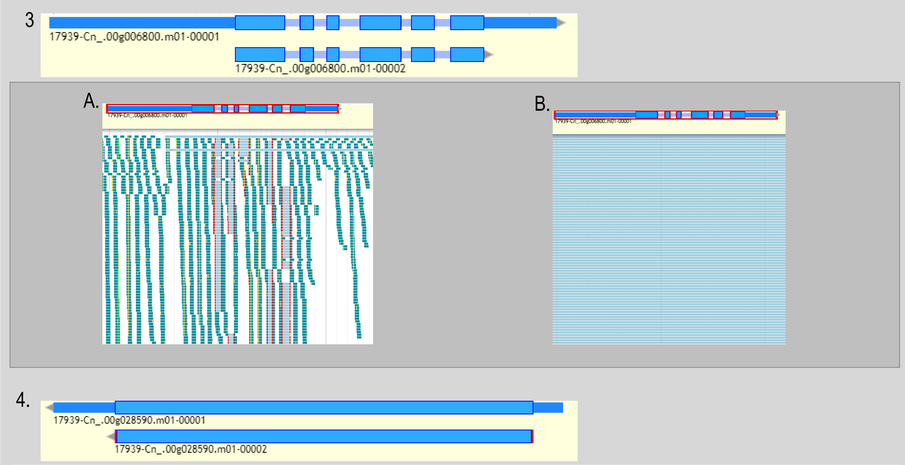



**Table 3: Ure G**

| Figure | Strains | Exon Problems |
|---|---|---|
| *A* | B15, B18, B40, B30, B43, B45, B58 H99S_Sc100_RM1-3 H99S_Sc100_T_RM1-3 | exons 1 and 6: longer |
| *B* | H99S_RM1-3 | undefined |

**Figure 3. Shows the revised PASA version and original genome of Urease Accessory Protein (Ure G)**

**Table 4: Urease Accessory Protein**

| Figure | Strains | Exon Problems |
|---|---|---|
| *A* | all | exon 1: not defined |

**Figure 4. Shows the revised PASA version and original genome of Urease Accessory Protein**

**Table 5: Strains observed on Apollo**

| Strain | Strain Names | Strain | Strain Names |
|---|---|---|---|
| B15 | replicate 1 | B43 | replicate 1 |
| | replicate 2 | | replicate 2 |
| | replicate 3 | | replicate 3 |
| B18 | replicate 1 | B45 | replicate 1 |
| | replicate 2 | | replicate 2 |
| | replicate 3 | | replicate 3 |
| B40 | replicate 1 | B58 | replicate 1 |
| | replicate 2 | | replicate 2 |
| | replicate 3 | | replicate 3 |
| B30 | replicate 1 | H99S | H99S_RM1-3 |
| | replicate 2 | | H99S_Sc100_RM1-3 |
| | replicate 3 | | H99S_Sc100_T_RM1-3 |

## Conclusion

Almost all of the genes have the first exon longer than the PASA predicted gene which means these greened could be longer than PASA predicted. Also, most of the genes have the last exon either missing or shorter than the PASA predicted genes. The evidence shows that the Cryptococcus neoformans genome needs to be further annotated.

## Current/ Future Research

Next semester will be the continuation of this project. Will annotate these genes further. In a wet lab students will perform experiments to figure out where exon 1 begins.

## Literature Cited

Brooks GF, Carrol KC, Butel JS, Moore SA, Mietzner TA: *Jawetz, Meinick, & Adelberg's Medical Microbiology,* 26th Edition; www.accessmedicne.com

McClelland E, Casadevall A, Eisenman H. 2007. Pathogenesis of Cryptococcus neoformans. In: Kavanagh K, editors. Insights in Medical Mycology. Springer Netherlands. p. 131-157.

## Acknowledgements

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