

# Influence of *SCP1* on *Cryptococcus neoformans* Virulence

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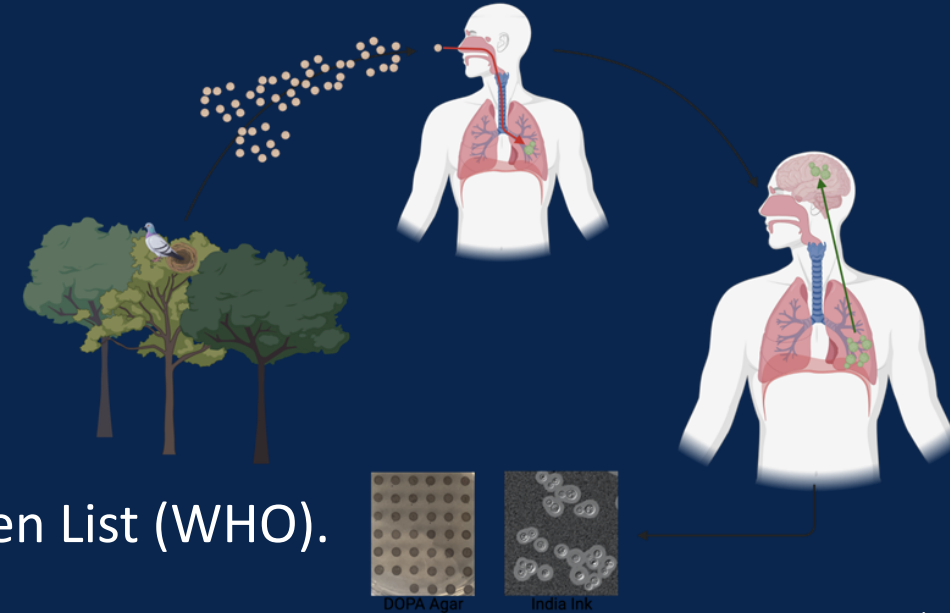
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# Background:

- Fungal pathogen
- Most affected: Immunocompromised individuals
- Critical priority pathogen on the Fungal Priority Pathogen List (WHO).
- Mortality rate: 41-61%
- Roughly 200,000 deaths annually
- Opportunistic pathogen
- Begins as pneumonia, crosses blood brain barrier, becomes meningoencephalitis
- Fatal without treatment.



# Current Antifungal Therapies:

- Treatments: Amphotericin B and Fluconazole.
- Amphotericin B- Toxic Fungicidal
  - Immediate treatment- IV infusion.
  - Binds to ergosterol- Makes cell membranes leaky.
  - Negative effects: nephrotoxicity, anemia, hypotension, etc.
- Fluconazole- Fungistatic
  - Lifelong treatment.
  - Prevents ergosterol synthesis.
  - Fungal antigens are becoming resistant.



# Research Goal

- Our goal is to identify a gene that can be targeted in the future for more effective anti fungal therapies.
- Long-term Plan:
  1. Identify gene
  2. Wildtype Strain
  3. Mutant strain
  4. Reconstituted Strains
  5. Virulence Assays



# SCP1 Gene

- Crypto's survival and virulence is reliant on adaptation
- Wildtype upper limit: ~ pH8
- This adaptation is due to many genes.
- Needed to adapt, survive, and become virulent.
- *SCP1* gene: SREBP Cap
- Regulates a transcription factor that regulates sterol biosynthesis
- **Research Question: Does the *SCP1* gene influence pH adaptation and virulence in *Cryptococcus neoformans*?**



# 1. Identify- Mutant Library Screening

- Dr. Alspaugh (Duke) identified a number of genes involved in pH adaptation.
- Mutant Library Screening:
  - *SCP1* is one gene.
  - RIM101 Pathway
    - pH regulatory pathway
  - SRE1 Pathway
    - Sterol homeostasis pathway
    - Regulated by *SCP1*

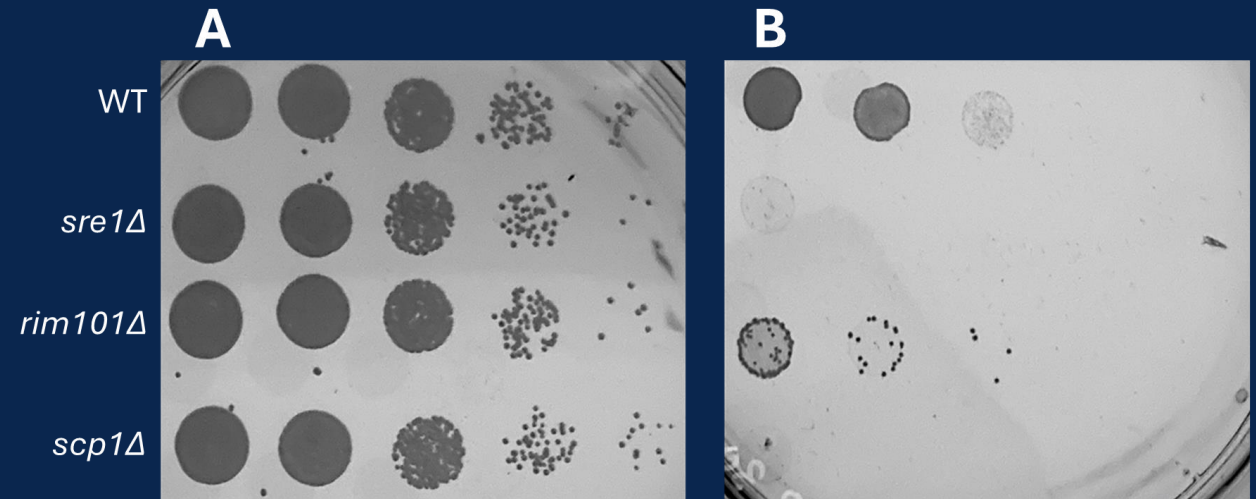


Image Source: Price Labs



## 2. Wildtype Strain

- Wildtype- KN99 $\alpha$ 
  - Cells Viable on YPD and pH8.



**YPD**

Image Source: Price Labs





# 3. Mutant Strain

- *SCP1* deleted via biolistics.
  - Lack of growth indicates successful knockouts

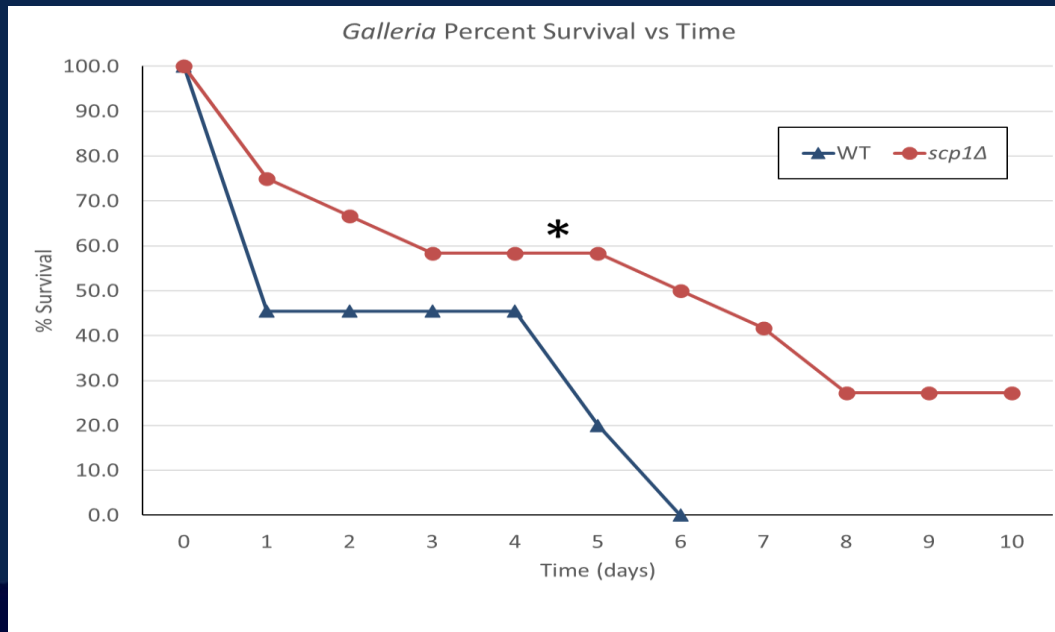
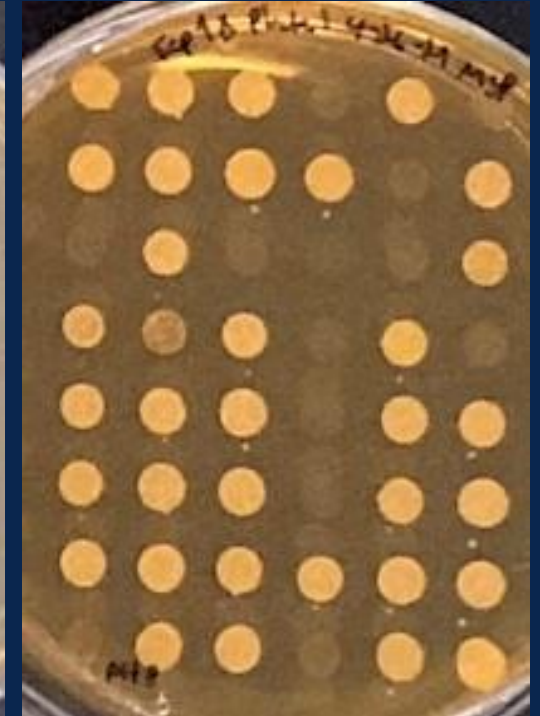


Image Source: Price Labs



YPD



YPD pH8

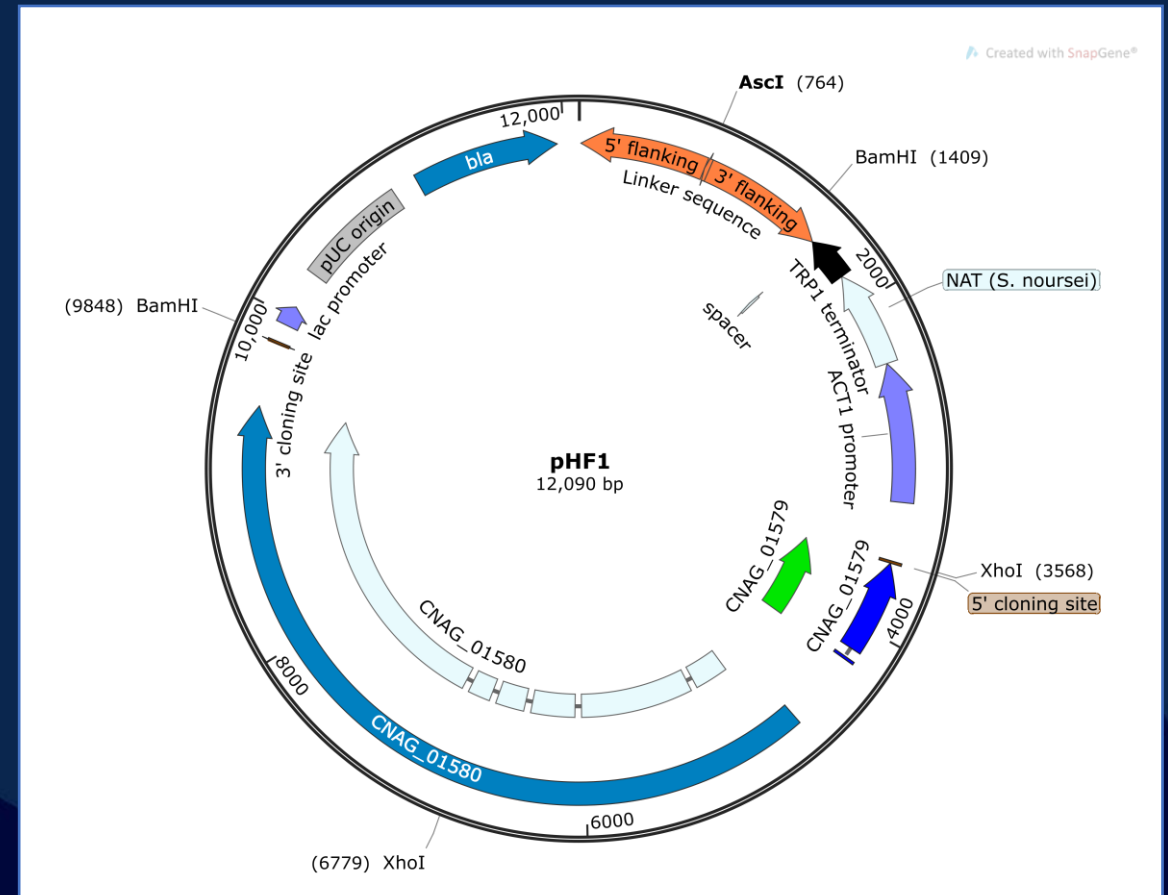
Image Source: Price Labs





# 4. Reconstituted Strain

- Reintegrate DNA:
  - Should have same phenotype as wildtype
- 1. Electroporation:
  - Unsuccessful
  - DNA would not integrate



# Biolistic Bombardment

- 2. Gene Gun:
  - Physically inserts DNA into cells.



Image Source: Carleigh Warsings



## 4. Reconstituted

- Next Step- Growth phenotype assay
  - pH8, 1.5M NaCl, CoCl<sub>2</sub>

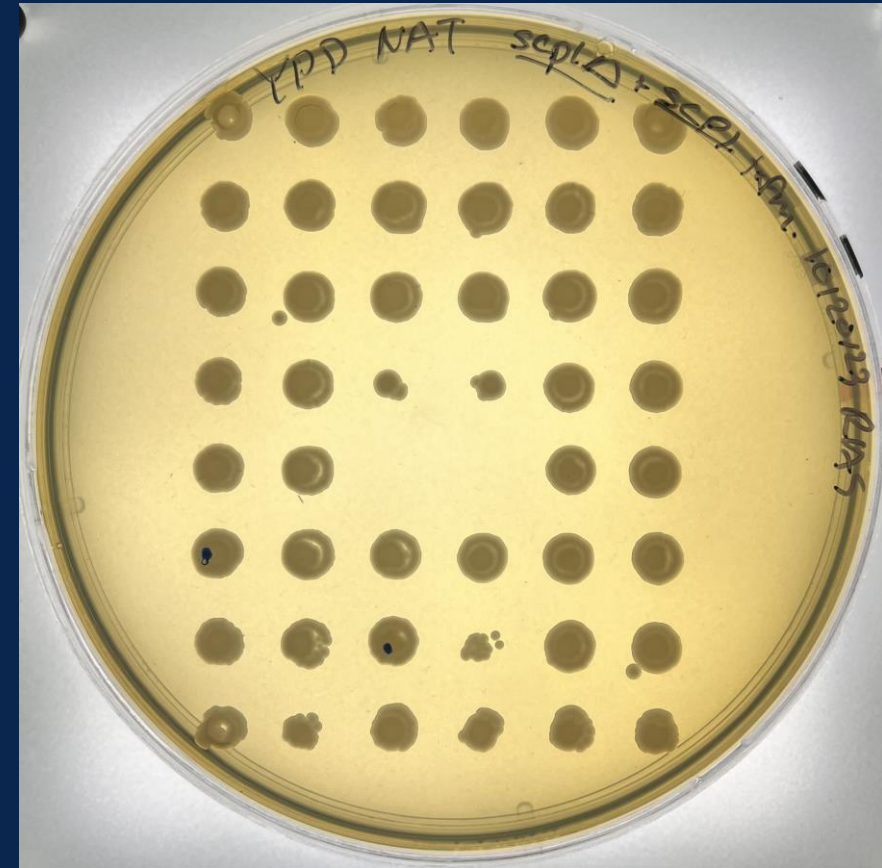


Image Source: Price Labs



# 5. Virulence Assays

- Invertebrate model
  - *Galleria* moth larvae
- Murine model (mice)
  - IACUC approved
  - Mimics natural infection



Invertebrate Model (*Galleria*)



Inhalation Model (mouse)

Image Source: Price Labs



# Future work

- Growth Phenotype assay
  - pH8, 1.5M NaCl, CoCl<sub>2</sub>.
- Virulence assay
  - Invertebrate and mouse models.
- QPCR data.
- Evaluate fluconazole sensitivity.



# Questions?

