

Activity of Plant-Origin Compounds on Fungal Morphogenesis

Marcia Lee

Medical Laboratory Science (MLS) Program

Miami University

Contents

Background:

Part I: Plant-Origin Compounds

Part II: Fungal morphogenesis

Hypotheses: Inhibition of fungal germination

Experimental Design: Morphogenesis Assays

Future Implications: Antimycotic treatment

Plant-Origin Compounds

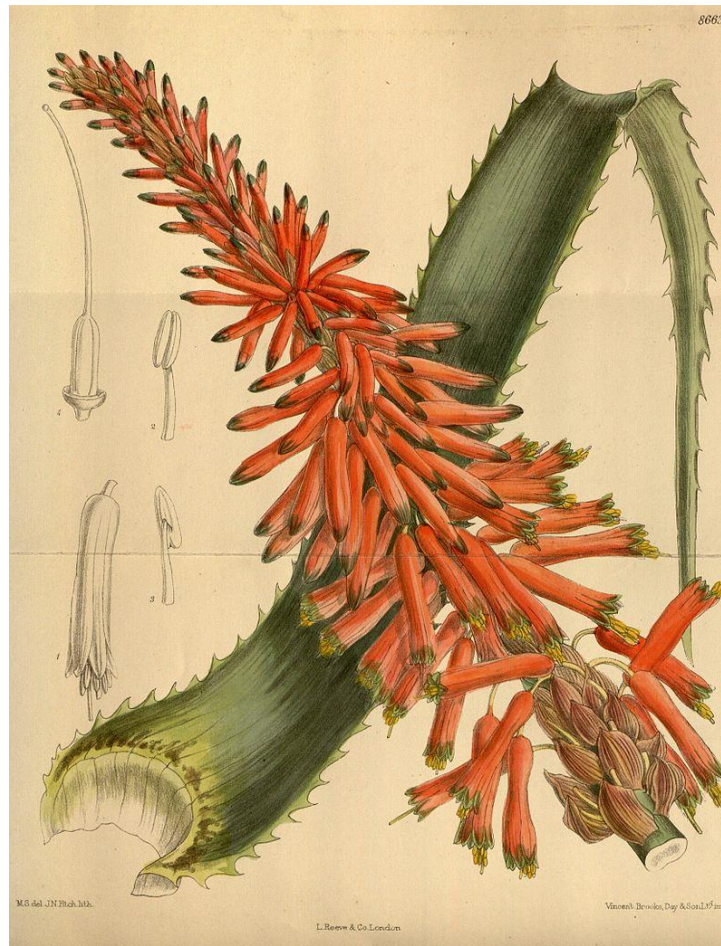
- World Health Organization (WHO):
85% of the world population use
plants/derivatives to treat ailments
- *Aloe* species
 - perennial
 - *A. arborescens* “tree-like”, the
“gold-tooth” aloe



Aloe arborescens, full bloom" Jimenez Nursery, Inc. Growers of specimen trees and palms.

Plant-Origin Compounds include:

- anthraquinones
 - aloe-emodin
 - chrysophanol
- pyrones
 - aloenin
- chromones
 - aloesin





Belk Greenhouse - Western Campus

Origin: South Africa

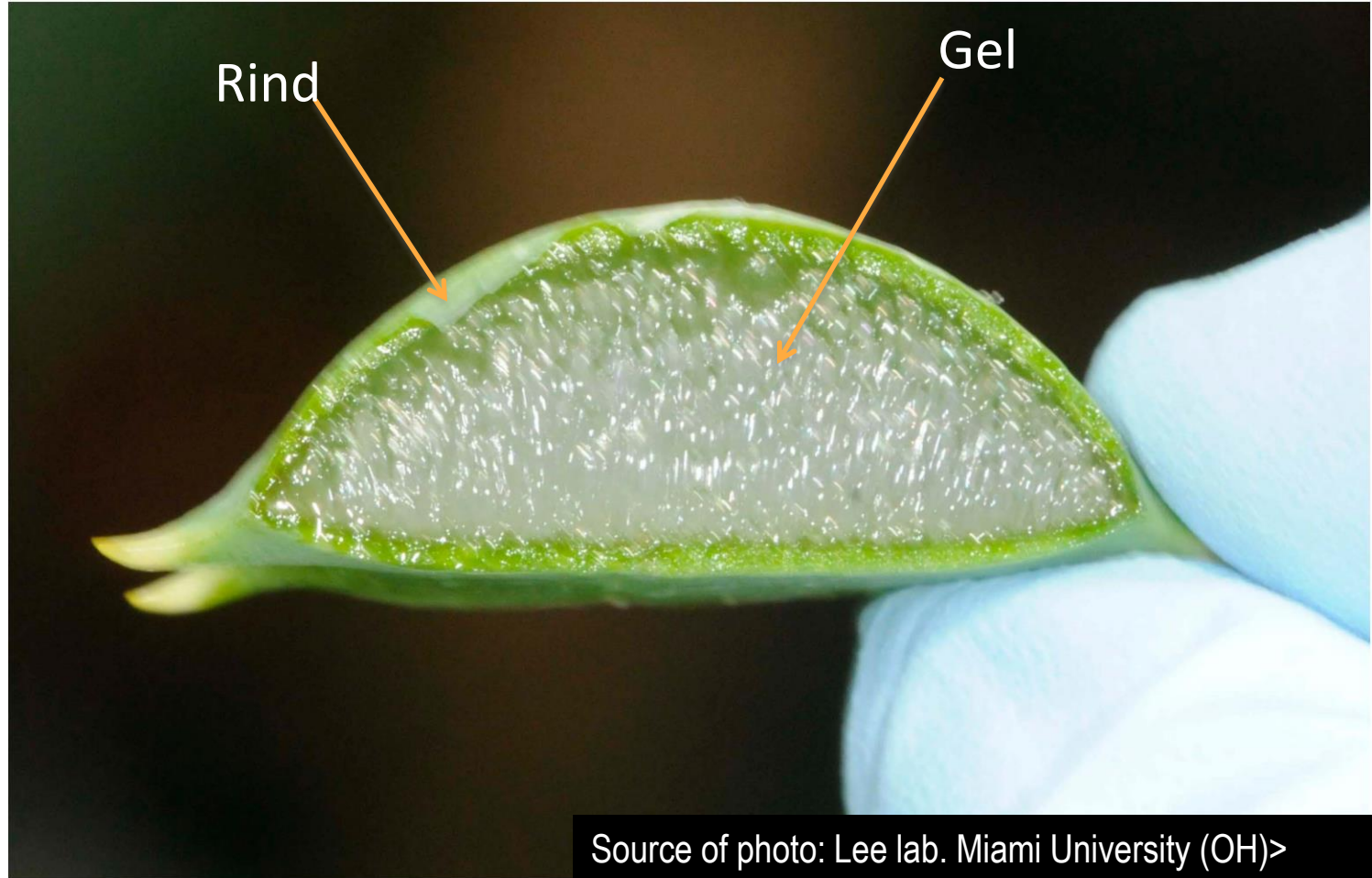
Physical Characteristics:

- Thick, spike-lined green leaves
- Red flower - 2 m

Grown in a fungicide-free greenhouse



Cross section of an *Aloe* leaf



Source of photo: Lee lab. Miami University (OH)>

Contents

Background:

Part I: Plant-Origin Compounds

Part II: Fungal morphogenesis

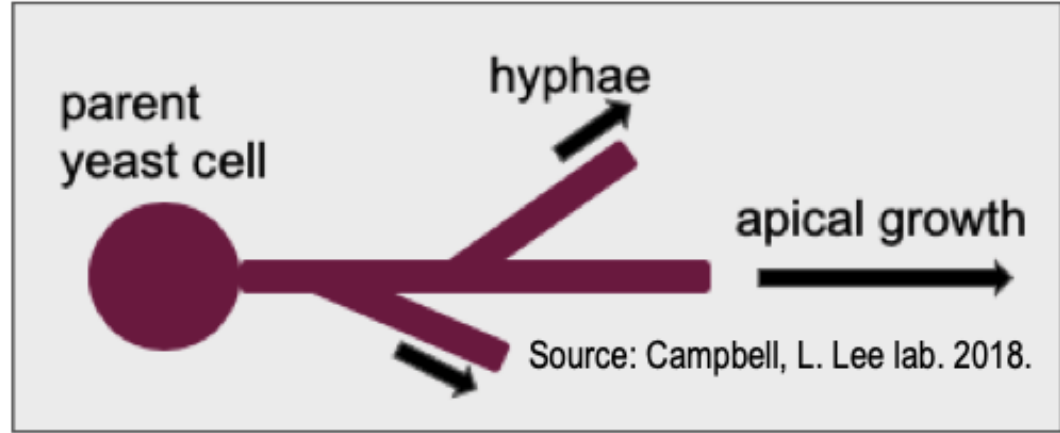
Hypotheses: Inhibition of fungal germination

Experimental Design: Morphogenesis Assays

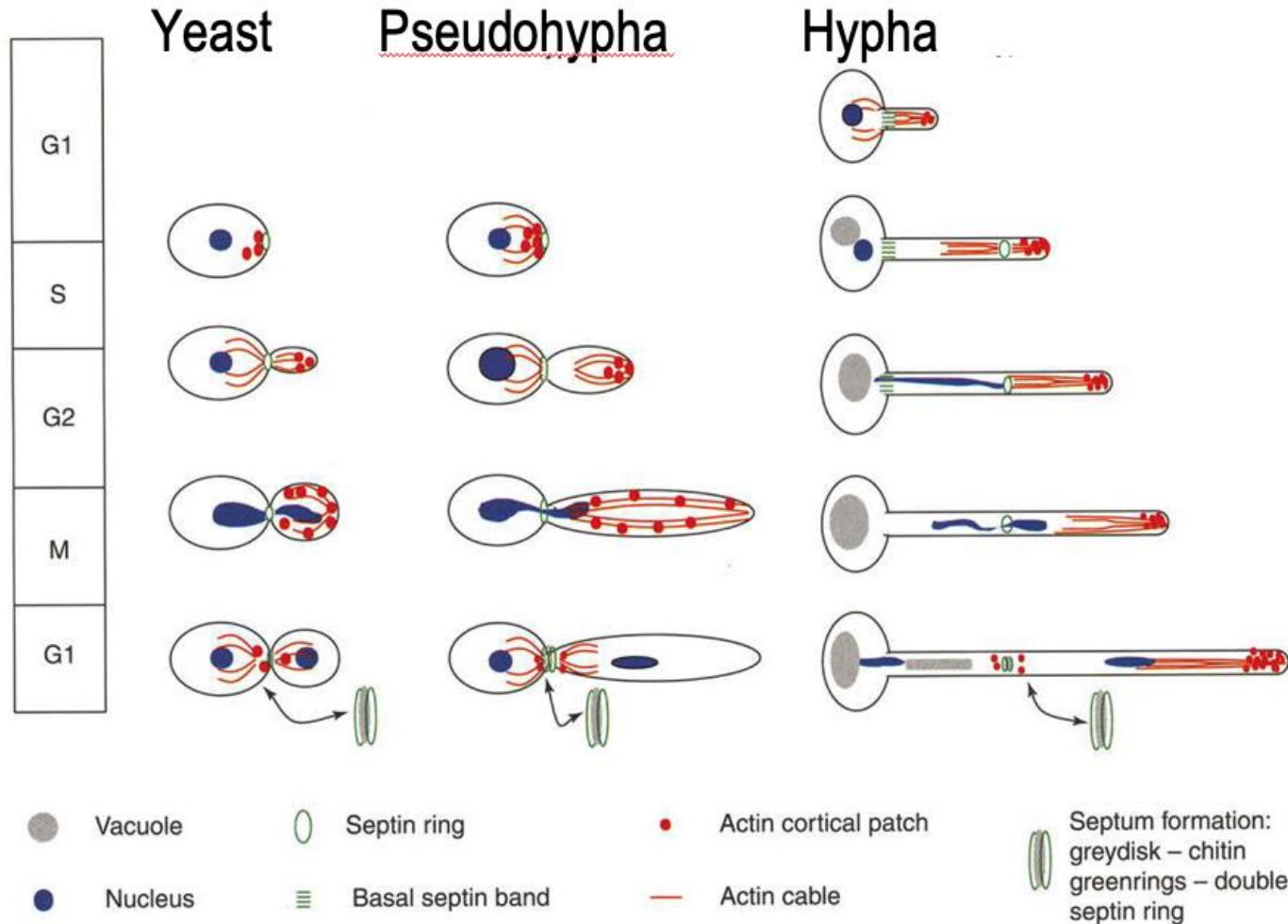
Future Implications: Antimycotic treatment

Fungal morphogenesis

- Kingdom Fungi
 - 99,000 known species
 - Yeasts, molds, mushrooms
- Fungi are:
 - eukaryotic
 - heterotrophic
- Growth
 - yeast forms: unicellular, may form buds
 - mold forms: form filaments (hyphae)
 - apical growth
 - dichotomous branching



Fungal morphogenesis



Sudbery, P. et al. 2004.
"The distinct
morphogenic states of
Candida albicans."

Preparation of fungal
inoculum

Preparation of treatment (either chemical compound
that we isolated from *Aloe* plant OR purchased
chemical from Sigma-Aldrich Chemical Company)



Morphogenesis assay



Microscopic detection of
morphogenic forms



Statistical analysis

The fungus = *Candida albicans* Traits

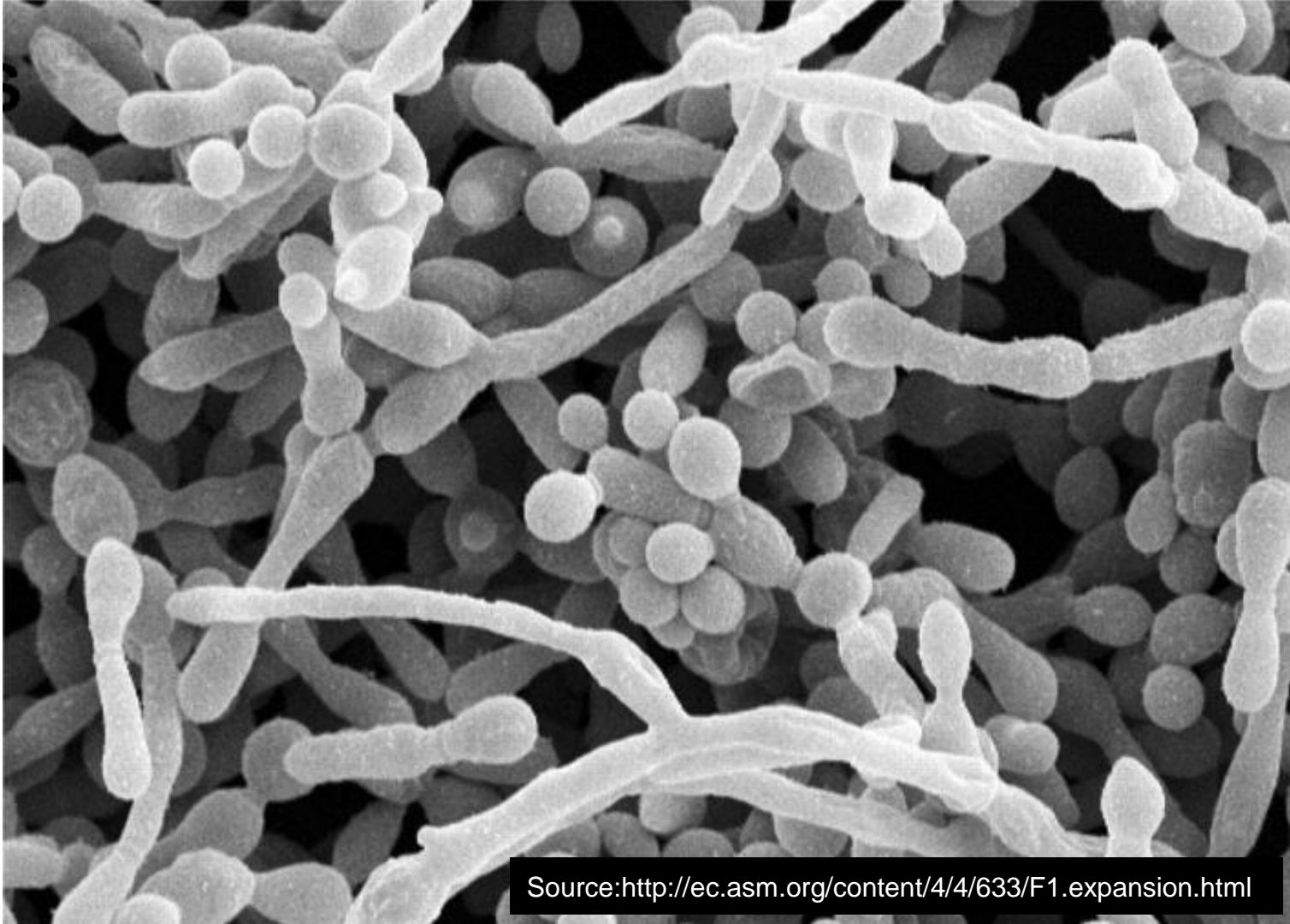


- part of normal, mammalian (including human) microbial flora. Is a ubiquitous, commensal fungus
 - oral cavity, urogenital, gastrointestinal flora
 - shape: oval, size: ca. 10-12 μ widest dimension
- can grow (form) multiple morphologies both in vivo and in vitro
 - unicellular yeast
 - multicellular, true hyphal form (singular: hypha, plural: hyphae)
 - pseudohyphal form (singular: pseudohypha, plural: pseudohyphae)

Fungal morphogenesis

- Burn wounds, thrush
- Broad-spectrum antibiotics
- Compromised immune system

Candida
biofilm
10µm



Source: <http://ec.asm.org/content/4/4/633/F1.expansion.html>

Preparing Aloe extract and isolating its compounds

- Harvest gel from *Aloe arborescens*
- 0.2 μ m filter and centrifuge gel, harvesting supernatant into 10 mL aliquots
 - 1.0 centipoise (CP) viscosity
- Rehydrated with RO water

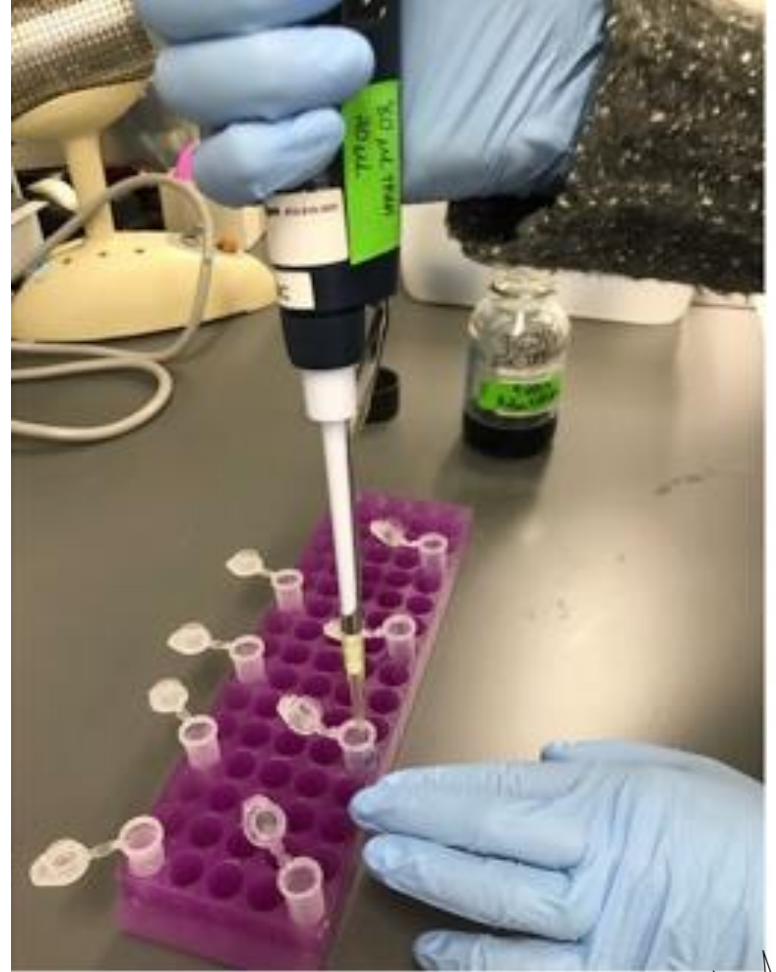
Or

- Rehydrate Sigma-Aldrich Company compound



Data Collection

- Assays stained with Lactophenol aniline blue (LPAB)
- Microscopy:
Differential Interference Contrast (DIC) system



Experimental set-up

C. albicans inoculum
plus 12.5% peptone



Treatments

Controls



- Morphogenesis Assay
 - Includes seven experimental treatments
 - a. whole *A. arborescens* extract
 - b. compound 'A' isolated from *Aloe arborescens*
 - c. compound 'A-SA' purchased from Sigma-Aldrich Company
 - d. compound 'B' isolated from *Aloe arborescens*
 - e. compound 'B-SA' purchased from Sigma-Aldrich Company
 - f. combination of A and B
 - g. combination of SA and SB
- Five replicates per treatment
- 200 cells are counted for each replicate of each of the treatments

- We will examine four replicates for each experiment
- Each cell will be examined microscopically and determined that the treatment resulted in formation of one of the following eight **responses**:
 1. No hypha (GT = synonym for hypha), no pseudohypha (PH), no buds (B)
 2. GT only
 3. PH only
 4. B only
 5. GT + PH
 6. GT + B
 7. PH + B
 8. > 1 GT

Our hypotheses are:

- 1) Exposure of *Candida albicans* cells to each of the seven treatments results in statistically significant reductions in formation of hyphae compared to non-exposed cells.
- 2) Exposure of *Candida albicans* cells to b. and c. treatments results in greater reductions in formation of germ tubes compared to treatments d. and e.
- 2) Exposure of *Candida albicans* cells to treatment a. results in the greatest reduction in germ tubes as compared to the other 6 treatments.