How antifungal drugs kill fungi and cure disease
A groundbreaking product was released in Japan that actually eats Candida-yeast and brings your inner ecology back to normal, all without having to go on special diets that are impossible to follow. The secret behind this product’s effectiveness is the micro-encapsulation process that gets live lactic acid producing bacteria safely past the acidic environment in the stomach. These oxygen-loving bacteria go to work creating an environment that is unfriendly to anaerobic problem organisms such as Candida. Many people who have suffered for years and tried everything on the market with little to no success report amazing results in the first few days. Reference reveals source to buy wholesale. $5.00

Do you suffer from depression, anxiety, irritability, heartburn, indigestion and bloating, constipation, foul breath, rashes, lethargy, some food and environmental allergies, acne, dry flaky skin, jock itch, or vaginal infections?

If you do, there is a good chance you could have an overgrowth of Candida and other unfriendly bacteria flourishing in your intestinal tract.
Types of fungal disease

- Skin infections: e.g. foot fungus (usually smelly but not life threatening, sometimes becomes serious), ring worm
- Mucosal infections: oral or vaginal (range from annoying to painful to very difficult; uncomfortable but rarely life threatening)
- Systemic infections: fungus in the blood and tissues (immunocompromised population, usually life threatening)
Onychomycosis: foot fungus
Oral Candidiasis
Systemic Infection

- Susceptible population: abdominal surgery, cancer chemotherapy, bone marrow transplant, organ transplants, other immunotherapy, other immune-compromising disease

- High mortality: those people that get systemic infection are already sick; current drugs are not effective
Fungi are relatively closely related to humans.

Within the Eukarya, the 5-kingdom system distinguishes Animals, Fungi, Plants & Protists. It can easily be seen that Protists are a highly diverse, paraphyletic group, while Animals, Plants and Fungi are all very closely related.

Note that plant plastids (chloroplasts) and mitochondria are more closely related to bacteria than to the nuclei of the cells in which they live.
Anti-microbial drugs

- Specificity (no side effects)
- Activity throughout the body
- Broad spectrum
- Kill microbes, not just prevent growth
- No drug-drug interactions
- Low cost
Current anti-fungal drugs

- Different classes of drugs target the plasma membrane, sterol biosynthesis, DNA biosynthesis, and β-glucan biosynthesis.
- Fungal membranes and sterol biosynthetic enzymes are different enough from ours that these agents can kill fungi but not us.
- Fungi make β-glucan, we don’t, so drugs that target β-glucan biosynthesis have low side-effects.
Mechanism of action (I)

- Cell wall biosynthesis
- DNA Synthesis
- Sterol biosynthesis
- Cell Membranes
Azole drugs target the fungal-specific synthesis of membrane lipids.

Amphotericin inserts preferentially into fungal membranes and disrupts their function.
Mechanisms of action (III)

Echinocandins target synthesis of β-glucan, a fungal-specific cell wall molecule.

Candida albicans on human epithelium Source: Holland/Özel, Robert Koch-Institut Berlin
Mechanism of action (III)

5-fluorocytosine targets fungal-specific DNA replication
What’s missing in antifungal therapy?

- **Specificity (no toxicity)**
  - Activity throughout the body

- **Broad spectrum**
  - Kill microbes, not just prevent growth

- **No drug-drug interactions**
  - Low cost
Molecular dissection of fungal infections

How can we identify new targets for broad-spectrum, safe, effective drugs?
When macrophages meet fungi

VS.
Immune cells recognize and respond to fungal surface molecules

- Two main fungal-specific molecules are β-glucan and mannan, chains of sugars linked in particular order.
- Immune receptors bind to these molecules and begin a choreographed immune response.
- A productive immune response is tiered: first immune cells signal an invasion and recruit more immune cells to the site of infection, then these cells kill the fungus and stimulate a long-lived response that protects against future infection.

The Macrophage
Body's Radar

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The image contains a slide with text and a diagram depicting immune cells and fungal cells. The text explains how immune cells recognize and respond to fungal surface molecules, focusing on the roles of β-glucan and mannan, and the tiered immune response.
Recognition of β-glucan stimulates the anti-fungal immune response

- Phagocytosis of the fungus (leads to killing)
- Activation of killing functions
- Production of attractive and activating signaling molecules
- Priming of the adaptive (memory) arm of the immune system to develop fungal-specific antibodies and T-cells
β-glucan, it cures what ails you

DO YOU EAT RIGHT, HAVE NO STRESS, EXERCISE REGULARLY AND SLEEP 8 HOURS A NIGHT TO KEEP YOUR IMMUNE RESPONSE IN PEAK CONDITION?

FOR WHEN YOU CAN'T – 30 MG BETA GLUCAN!
Fungi are just like M&Ms!

1\textsuperscript{st} Layer: Colored Candy Paint
Fungi are just like M&Ms!

2nd Layer
White Candy Shell
Fungi are just like M&Ms!

3rd Layer Chocolate Center
Fungi are just like M&Ms!

Colored Candy Paint (Mannan)

Exposed White Candy Layer (β-glucan)
Layered architecture masks most $\beta$-glucan from the immune system

- mannann
- $\beta$-glucan
β-glucan is present everywhere but is mostly invisible to immune molecules.
Wildtype yeast have very little exposed $\beta$-glucan

Colored Candy Paint (Mannan)

Exposed White Candy Layer ($\beta$-glucan)
Disruption of the cell wall exposes β-glucan and fungi are recognized better.
Recognition of $\beta$-glucan stimulates the anti-fungal immune response

- Phagocytosis of the fungus (leads to killing)
- Activation of killing functions
- Production of attractive and activating signaling molecules
- Priming of the adaptive (memory) arm of the immune system to develop fungal-specific antibodies and T-cells

**BUT:** The fungi masks its $\beta$-glucan under a candy coat to block these responses
Is there a drug that can unmask fungi?
Caspofungin causes exposure of $\beta$-glucan at sub-MIC concentrations
Caspofungin also causes β-glucan exposure in filaments
Is there a clinical relevance?

- We still have not tested this in a mouse model of fungal infection but we hope that drugs that can “unmask” fungi will lead to better immune responses against the fungi.
Is there a clinical relevance?

Many “targeted” drugs have more than one activity which contribute to their effectiveness (e.g. Gleevec, fluconazole) or toxicity (Vioxx)

Most drugs, including caspofungin, do not have homogeneous tissue distribution
Variability in tissue distribution of caspofungin

**TABLE 3. Mean concentrations and amounts of radioactivity in the tissues of rats receiving a 2.0-mg/kg i.v. bolus of [3H]caspofungin**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean ± SD concn (µg eq/ml or µg eq/g)</th>
<th>(mean amt as % of dose)</th>
<th>2 hour tissue distribution</th>
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</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>6.1</td>
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<tr>
<td>Red blood cells</td>
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<tr>
<td>Skeletal muscle</td>
<td>4.5</td>
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<tr>
<td>Fat</td>
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<tr>
<td>Skin</td>
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<tr>
<td>Heart</td>
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<tr>
<td>Lung</td>
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<td></td>
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<tr>
<td>Kidney</td>
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<td>Spleen</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Small intestine</td>
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<tr>
<td>Large intestine</td>
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<td>Brain</td>
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<td>Lymph nodes</td>
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<tr>
<td>Eye</td>
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</tr>
</tbody>
</table>

Note: n = 3 rats per time interval.
The take-home message

- There is a clear need for new anti-fungal drugs (and identification of new targets) to treat systemic infection
- The immune system recognizes fungi based on conserved fungal molecules which can induce protective (anti-fungal) responses
- *Candida* may mask these molecules to prevent a full-blown immune response
- Masking may be a drug-targetable mechanism useful in combatting fungal infection
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