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HEALTH
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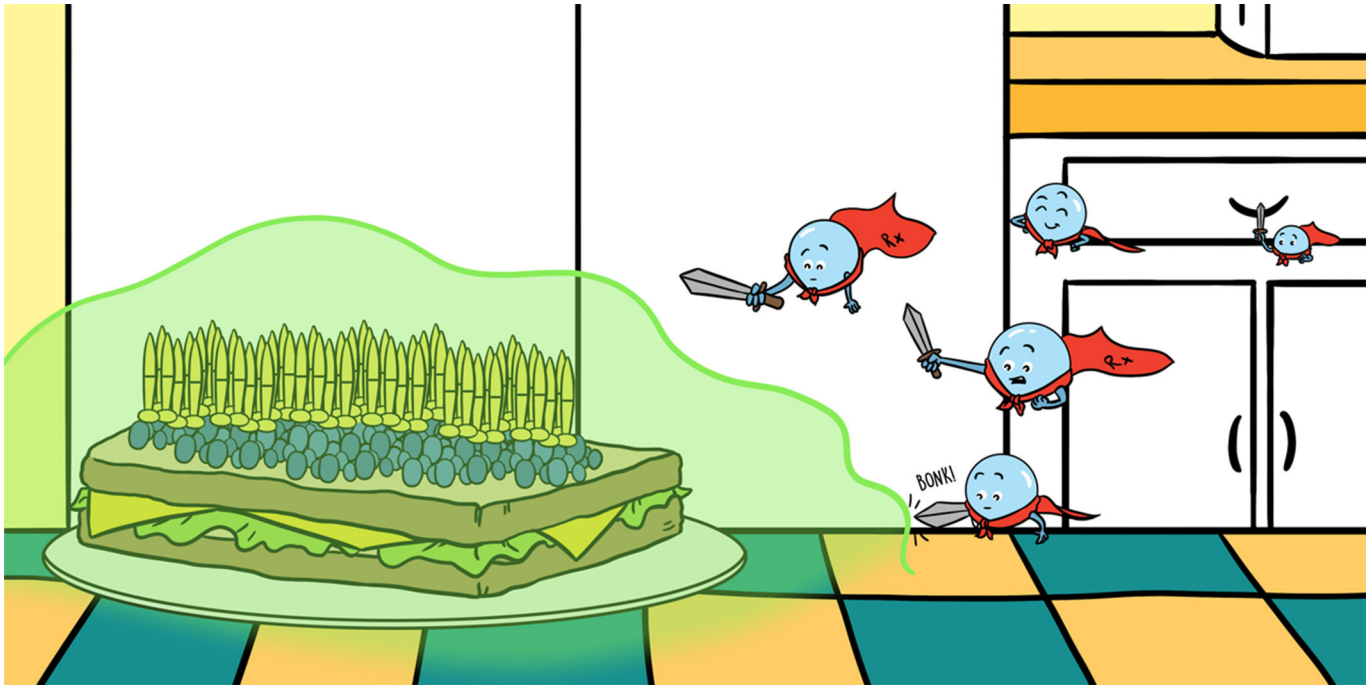

DISARMING A TRANSFORMER FUNGUS

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YOUNG REVIEWER:

OLIVER

AGE: 13

Fungi are some of the most important microbes on Earth. Fungi are very different from bacteria—their cells look more like animal or plant cells. Most fungi play important roles in nature and do not cause disease to humans, but the ones that do cause disease are very difficult to get rid of. Antibiotics that can kill fungi are known as antifungals. Very often, these drugs eventually stop working because fungi can become resistant to them! Fungi also form biofilms, which are communities of cells living together, surrounded by a protective shield. The fungus *Candida albicans* does not usually cause disease, but sometimes it can change shape from an oval cell known as a yeast, to a long cell called hyphae, which helps *C. albicans* invade and damage organs and form strong biofilms. Here, we explain a new strategy to stop *C. albicans* from causing disease, by stopping its shape shifting!

WHAT ARE FUNGI?

Fungi are some of the most interesting and amazing organisms on the planet. They are found everywhere, including in our bodies! Fungi mainly play the role of decomposers in the environment, breaking down organic matter such as dead animals and plants. Interestingly, the largest organism on earth is believed to be a fungus in the Blue Mountains in Oregon! We also eat fungi in the form of mushrooms and use them to make foods like bread, cheese, and yogurt. Fungi live happily in and on our bodies and are part of our normal microbiota, which is the word for all the microbes found in and on the body. Usually these fungi are not harmful, but sometimes they can cause diseases in humans, other animals, and crops. Fungi are very different from bacteria and viruses. Fungi are eukaryotic, meaning their cells have organelles, such as a nucleus and mitochondria, which bacteria and viruses do not have. Fungi might seem like plants to some people, and a long time ago we used to think they *were* plants, but we now know that fungi are more closely related to us!

WHO GETS FUNGAL INFECTIONS?

IMMUNE SYSTEM

The defense system used by our bodies to protect us from bad microbes.

IMMUNOCOMPROMISED

A person with a weak immune system.

BIOFILM

A community of microbes living close together, attached to a surface, and protected from the environment by a layer of protective substance.

The **immune system** is the body's way of protecting itself from bad germs. The immune system uses many different strategies to fight and kill troublesome invaders. If you become **immunocompromised**, meaning you have a weak immune system, you may not be able to protect yourself from bad microbes. Most fungi only infect people who are immunocompromised. Fungi also love growing on plastics and other materials! These surfaces allow the fungi to form very strong **biofilms**, which are communities of fungi living under a protective shield that blocks the immune system from attacking it and also prevents antifungals from killing the fungal cells living inside—think of the shield that protects Wakanda in the movies Black Panther and Infinity War. When fungi form these biofilms, the immune system and drugs cannot penetrate the shield! Fungi can also leave the biofilm and travel to other parts of the body to start a new infection. Imagine a fungus living in a biofilm that is growing inside the catheter (IV tube) in someone's vein. This catheter is connected to a highway in the body, the circulatory system. Once the biofilm gets too crowded, some fungal cells want to move out and find a new place to live, where they can start their own community, so they separate from the biofilm and get on the circulatory highway until they reach an organ where they can establish a new infection site.

Sadly, since fungi cause disease in people who already have weakened immune systems, and because the fungi can form biofilms to resist

the attack by antifungal drugs, they are able to cause high numbers of deaths in these immunocompromised individuals.

WHAT CAN WE DO WHEN FUNGI GO BAD? NOT MUCH

Treating diseases caused by this minority of "rogue" fungi can be very hard, because fungal cells are similar to our own cells! This means that anything that is toxic or bad to the fungi will also probably be toxic to our own cells. Fungi can mutate so that the drugs being used to fight them do not work anymore, or they can learn to "spit" the drugs out! In addition, fungi are much more resistant to antifungal drugs when they form a biofilm. These two problems, toxic drugs and "tough" fungi, have left us with very few options to treat infections caused by fungi [1, 2]. Something scary to consider is that there are some fungi out there that can kill 100% of the people they infect, because there is not a single antifungal drug that is effective against them! This is why it is very important for scientists to keep looking for new drugs and ways to fight fungal infections, and also to find ways to protect those who have weak immune systems.

C. ALBICANS

A fungus found in and on the human body that can sometimes cause disease in those with weak immune systems.

HYPHAE

A long form of a fungal cell, which helps it spread and find food. It can also cause damage to humans.

FILAMENTATION

A process in which fungi can change from yeast to elongated forms called hyphae.

CANDIDA ALBICANS, THE TRANSFORMER FUNGUS

Candida albicans is the most common of these problematic fungi. It is found in most people living on the skin, in the mouth, and in the intestines without causing any disease. But when a person is immunocompromised, is treated with antibiotics, or has implanted devices (catheters, pacemakers, and prosthetics) [3], ***C. albicans*** can transform into a disease-causing form! *C. albicans* is mostly found as a yeast, which is an oval-shaped cell that does not usually cause disease, but it can shape-shift into a long cell known as a **hyphae,** which allows it to invade organs in the body and form biofilms (Figure 1). This change from yeast to hyphae is known as **filamentation**. Think of the hyphae

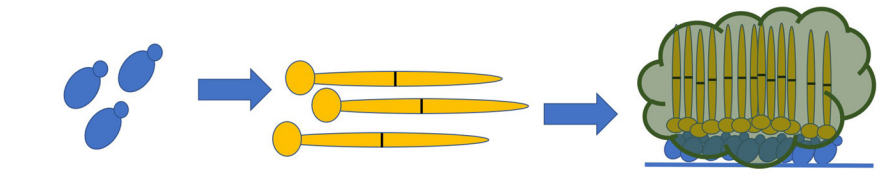

Figure 1

Figure 1

The different shapes of *C. albicans*. *C. albicans* can change its shape depending on where it is and what is going on around it. This allows it to go from non-harmful yeast to disease-causing hyphae and biofilms. Each of these forms has different properties.

as a jackhammer that allows the fungus to break through cells as it is invading a tissue. What is really neat about *C. albicans* is that it absolutely needs hyphae to be able to form biofilms, which makes hyphae formation a great target for drugs. If we are able to block the hyphae from forming, then the fungus will not be able to invade organs and will not be able to form biofilms, which means it stays as a yeast and does not cause disease!

HOW DO WE FIND NEW DRUGS TO KEEP *CANDIDA* FROM CAUSING HARM?

In order to find new drugs to help fight and/or prevent *C. albicans* infections we searched a chemical library consisting of 30,000 small molecules, looking for molecules that could stop *C. albicans* from forming hyphae [4].

After searching through all of the small molecules, we found a few that stop *C. albicans* from filamenting! Two of our most interesting small molecules are called compound 36 and compound 44. We found it interesting that these two molecules are related to each other, as you can see by comparing their shapes (Figure 2). We chose compound 36 for our experiments, because it was better at stopping filamentation. Since we wanted to make sure that compound 36 actually stopped the formation of hyphae, we did more experiments to test it. If you remember, we were looking for small molecules that could stop filamentation, but we also wanted to know if the molecules could stop biofilms from forming. To test this, we put *C. albicans* in liquid food called RPMI 1640, which makes *C. albicans* form biofilms, and added compound 36 at different concentrations in what it is called a **dose-response** assay. As you can see in Figure 3A, compound 36 was able to stop or prevent

DOSE-RESPONSE

Testing a drug/small molecule at different concentrations and measuring its effect. Generally, one would expect that the highest the concentration the biggest the effect.

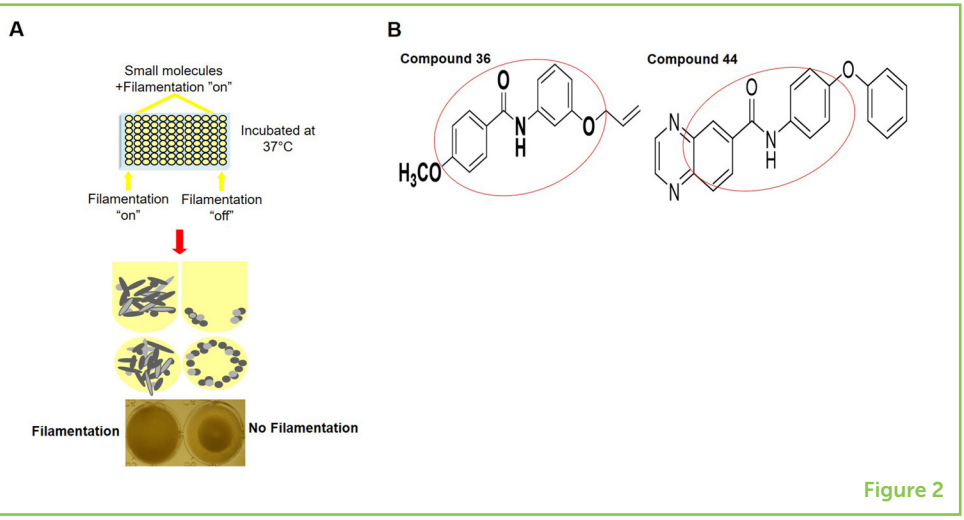

Figure 2

Small molecules that can stop *C. albicans* filamentation. **(A)** We screened 30,000 small molecules, looking for some that could stop *C. albicans* filamentation. **(B)** These are the chemical shapes of compound 36 and compound 44, two of the best small molecules we identified. These two compounds are related to each other, as you can see by the similar structure of the parts highlighted in red.

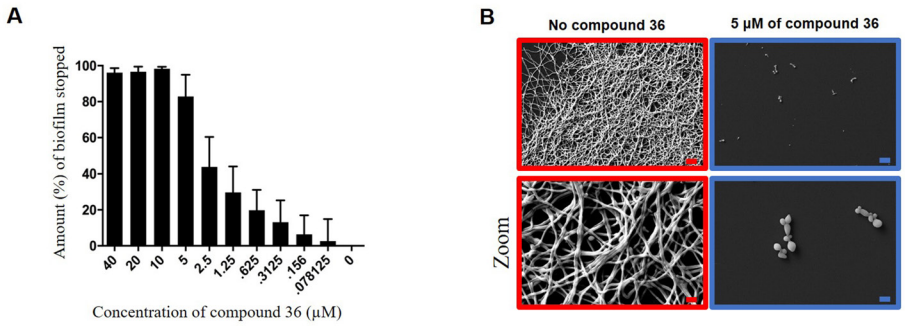

Figure 3

Figure 3

Compound 36 stops biofilms from growing. **(A)** *C. albicans* biofilms were grown with compound 36 at different concentrations (40–0.078 μM). Then we measured the amount of biofilm formation and compared it to the amount of biofilm formed in the absence of this compound. As seen in the graph, compound 36 stopped biofilms from forming by about 50%, even at 2.5 μM. **(B)** Scanning electron microscopy was used to look closely at the biofilms when they were grown by themselves or when they were grown with compound 36. When compound 36 was present, biofilms did not form.

biofilms from forming, even at very low concentrations (results in the graph are showing the percent of the biofilm that was stopped, so the higher the bar, the less of a biofilm there is)!

We also wanted to see if compound 36 had any effect on the shape of the cells, since we were looking for small molecules that could block the change from yeast to hyphae. In order to look at the cell shape, we used a tool called a scanning electron microscope, which allowed us to look at cell shape closely and at high definition (Figure 3B). In the absence of compound 36, *C. albicans* was able to form thick biofilms (left images), but when compound 36 was present, it was able to stop biofilms from forming (right images). Interestingly, the cells did not look like regular yeast, which are oval-shaped. The cells that were treated with compound 36 looked like they were trying to filament but were unable to do so!

WHY IS THIS WORK IMPORTANT?

Fungi are often ignored in the environment and in medicine, but they are very important members of all the communities they are part of. Fungi are found in our bodies, but not much is known about what they do in that setting. What we do know is that when they cause disease, it is very difficult to get rid of them. This is because we have very few drugs to successfully treat fungal infections. The strategy that we explored in this article is called anti-virulence, because it fights the bad microbe by taking away its weapons, which are called virulence factors. In the case of *C. albicans*, the virulence factor is the ability to change from yeast to hyphae, because this is what allows it to cause disease! Scientists have been using this anti-virulence strategy to treat infections caused by bacteria, but no one has previously done it for fungi. The great thing about anti-virulence strategies is that they just disarm the fungus without killing it. This way we keep our "frenemy" in check!

ORIGINAL SOURCE ARTICLE

Romo, J. A., Pierce, C. G., Chaturvedi, A. K., Lazzell, A. L., McHardy, S. F., Saville, S. P., et al. 2017. Development of anti-virulence approaches for candidiasis via a novel series of small-molecule inhibitors of *Candida albicans* filamentation. *MBio* 8:e01991-17. doi: 10.1128/mBio.01991-17

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CONFLICT OF INTEREST STATEMENT: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWER

OLIVER, AGE: 13

I am an active boy, enjoying out-door life. I especially like football and hunting.

AUTHORS

JESUS A. ROMO

I love to study fungi. They are fascinating organisms that can be found almost everywhere and play very important roles in those environments. My favorite fungus is *Candida albicans,* and I study its ability to shape shift into forms that give it the ability to cause disease by forming hyphae and biofilms. My work also involves trying to find new molecules that can prevent *C. albicans* from changing shape and can be used as new treatments for infections caused by this fungus. I also spend some of my time working on ways to communicate my science to the public through social media and outreach efforts as a means to educate and diversify the sciences. *jesus.romo@Tufts.edu

JOSE L. LOPEZ-RIBOT

I am a Professor of Biology at the University of Texas at San Antonio. For the last 30 years, ever since I started my doctoral studies in my native Spain, I have been studying *Candida albicans*. Throughout the years my laboratory has been involved in studies related to *C. albicans* cell biology (i.e., cell wall, filamentation, biofilms), immune responses (i.e., antibodies, vaccines), and drug resistance; but during the last few years our focus has been on the development of what we call "anti-virulence" approaches to treat infections caused by this fungus. We hope that 1 day in the near future this new approach can be used to treat patients suffering from these devastating infections.

ASHOK K. CHATURVEDI

I work on medically important fungal infections mainly, due to *Candida* spp., and *Cryptococcus* spp. As the incidence of fungal infections has increased in the last decades, mostly due to high mortality associated with systemic infections, especially in the case of immunocompromised patients, there is an urgent need to develop new pan antifungal drug and/or vaccine against medically important fungal infections. My primary research interests lie in fungal immuno-proteomics, antifungal drug development, and vaccine development against medically important fungal infections. Research work in our lab has generated significant and valuable contribution toward understanding and making novel antifungal therapy.

STEPHEN P. SAVILLE

I am a yeast geneticist by training and study the fungus *Candida albicans* and how it is able to cause disease in humans. My work mainly focuses on understanding the genetic strategies used by *C. albicans* to change shape and finding new drugs to treat fungal infections. I also use advanced techniques to understand this fungus from within and how it is able to turn on and off specific genes that allow it to adapt to different situations and environments.

CHRISTOPHER G. PIERCE

I study the ability of *Candida albicans* to form communities known as biofilms. This gives *C. albicans* the ability to resist treatment and makes the infections much more difficult to deal with. The main goal of my laboratory is to find new drugs that can be used to control and treat infections caused by this fungus.

[†]These authors have contributed equally to this work