The Great Escape:
How Urea Amidolyase Allows A Pathogenic Fungus To Escape The Immune System

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Abstract:
According to Rice University, 70% of people are affected by the infectious fungus *Candida albicans*. The immune system uses T and B cells to stop pathogens. People with suppressed immune systems, such as transplant patients, and AIDs or cancer patients, lack functional T and B cells, and rely on macrophages to destroy *Candida*. *Candida* can kill and exit macrophages due to an enzyme: urea amidolyase (UAL). While in the macrophage, an environmental change causes *Candida* to morphologically switch from a sphere to a structure with hyphae. UAL converts urea to ammonia (NH_3) and CO_2, creating an environment for hyphae to form, bursting the macrophage. The Greenfield SMART (Students Modeling A Research Topic) Team used 3D printing technology to model UAL. The biotin carboxylase (BC) domain uses energy from ATP cleavage to attach CO_2 to the swinging arm portion, or biotin carboxyl carrier protein (BCCP) domain. The BCCP domain swings across UAL, attaching CO_2 to urea forming allophanate in the carboxyl transferase (CT) domain. Allophanate moves to the allophanate hydrolase (AH) domain, hydrolyzing the allophanate into CO_2 and NH_3. Increases in CO_2 and NH_3 cause hyphae to form, destroying macrophages and allowing *Candida* to spread. Researchers could block UAL's active sites to prevent *Candida*'s macrophage-killing shape change, preventing systemic candidiasis without damaging human cells. Supported by a grant from NIH-CTSA.

A. Systemic Candidiasis Affects People with Weakened Immune Systems:

Oral thrush[1]

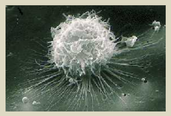
Skin rash[2]

Diaper rash[3]

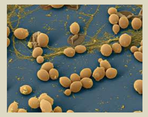
Normally, the human immune system uses white blood cells called T and B cells to fight pathogens. These cells do not function as well in people with weakened immune systems, such as children who have recently had a transplant, people with AIDS, and cancer patients. People with weakened immune systems must then rely on macrophages, another type of white blood cell, to fight pathogens. A common fungus, *Candida albicans*, can cause common, easy-to-treat diseases, such as oral thrush, vaginal and local yeast infections, and diaper rash. However, *Candida* can also cause systemic candidiasis, a potentially lethal infection that spreads throughout the body and is difficult to treat.

B. How Candida Escapes From Macrophages:

Step 1: When any foreign agent, such as *Candida albicans*, infects an organism, macrophages engulf and break down the pathogen.



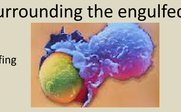
Macrophage[4]



Candida albincans[5]

Step 2: Once inside the macrophage, *Candida* adjusts its metabolism to produce large amounts of urea. An enzyme called Urea Amidolyase (UAL) converts this urea into ammonia (NH_3) and carbon dioxide (CO_2). The increased concentrations of NH_3 and CO_2 change the internal macrophage environment surrounding the engulfed *Candida*.

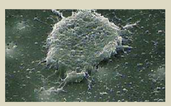
Macrophage engulfing *Candida*[6]



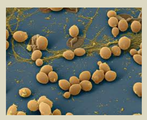
Step 3: Increases in $NH_3$and CO_2 signal the creation of dagger-like projections called hyphae. These projections kill the macrophage and allow the *Candida* to escape from inside.

Candida in the hyphal stage[7,8]

Step 4: The *Candida* is now free to continue colonizing the host organism's body. Ultimately this could lead to fatal systemic candidiasis.



Dead macrophage[9]

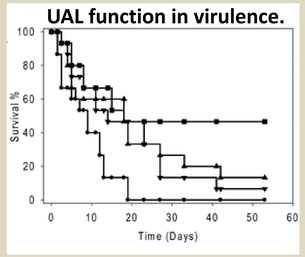


Candida albincans[5]

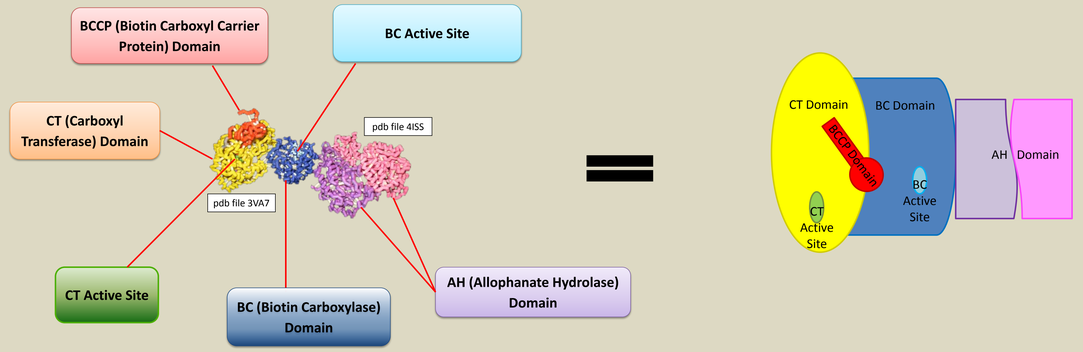
C. How Do We Know UAL Makes A Difference?

In an experiment, mice were injected with wild type *Candida albicans* (• in image to the right[10]). Over time, survival rate of the infected mice was calculated.

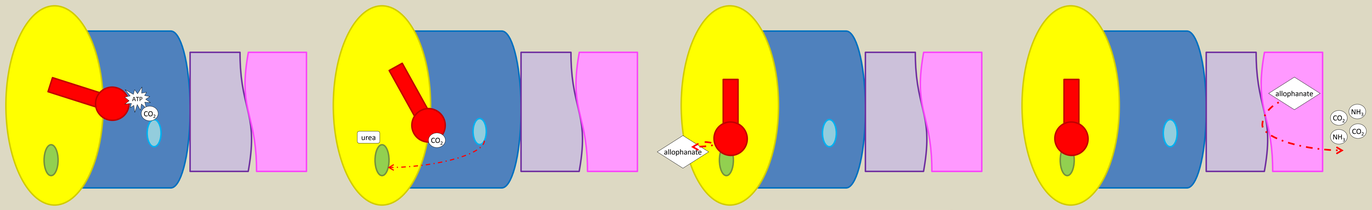
Similarly, mice were injected with *Candida* lacking the gene for UAL, called Dur 1, 2. This null mutant form is referred to as KWN6 (▪). Candida lacking Dur 1, 2 cannot make UAL. The survival rate of the mice infected with KWN6 was also calculated. The mice infected with KWN6 had the highest survival rate (about 50%) because these *Candida* could not make hyphae to break out of the macrophages.



D. The Four Domains of Urea Amidolyase (UAL)



E. How UAL Functions to Increase CO_2 and NH_3 Inside the Macrophage



1. The **BC domain** utilizes energy released from splitting ATP to attach CO_2 to the BCCP swinging arm component.

2. The **BCCP domain** swings across UAL to attach the CO_2 to urea in the CT domain.

3. The **CT domain** forms allophanate, which then travels to AH domain.

4. The **AH domain** has an active site that hydrolyzes allophanate into two CO_2 molecules and two NH_3 molecules, creating an environment for the *Candida* to form hyphae.

F. The Future of Urea Amidolyase in Preventing Systemic Candidiasis

In the future, targeting urea amidolyase could decrease occurrences of systemic candidiasis and related casualties. Future research could possibly find a method of preventing hyphae from being produced and thus not allowing the fungi to escape the macrophages. A method of inhibiting one of UAL's domains without interfering with human active sites could cause UAL to no longer function. Thus, people with compromised immune systems would be less susceptible to developing systemic candidiasis.

1<http://healthyjen.hubpages.com/hub/Oral-Thrush-Symptoms-and-Treatment> 2<http://medicalpicturesinfo.com/wp-content/uploads/2011/09/Candida-Skin-Fungus-2.jpg> 3<http://3.bp.blogspot.com/-8NikKo5yAww/T1FQzpqJI1I/AAAAAAAACfs/DFfhY1X41Sw/s400/Diaper_Rash-3.jpg> 4<http://www.thepigsite.com/pighealth/article/142/porcine-reproductive-and-respiratory-syndrome-prrs> 5<http://en.wikinoticia.com/lifestyle/beauty/86867-candidiasis--caprylic-acid> 6<http://www.sciencephoto.com/subject/Human%20Body/immune%20system/cell%20attack> 7<http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab9/chlamydo.html> 8<http://curezone.com/blogs/fm.asp?i=1993854> 9<http://www.thepigsite.com/pighealth/article/142/porcine-reproductive-and-respiratory-syndrome-prrs> 10<Navarathna DH, Lionakis MS, Lizak MJ, Munasinghe J, Nickerson KW, Roberts DD. Urea amidolyase (DUR1,2) contributes to virulence and kidney pathogenesis of *Candida albicans*. *PLoS One*. 2012;7(10):e48475>

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