

Trehalose provides sustained energy and is a stress protectant for proteins and membranes

By J. C. Spencer

Research mounts that trehalose acts as a stress protectant for plants and animals, proteins and membranes. Pathogen Research Show Trehalose Pathway as a Selective Fungicidal Target for Use in Antifungal Development. Trehalose has been found to play diverse roles, from energy source to a stress protectant. Improving stress tolerance and yield in crops are major goals for agriculture. Trehalose research in plants opens new doors for better production. The Endowment for Medical Research continues to be your educational resource for sugars that are beneficial to better health of plants, animals, and humans. Here are more reports of ongoing trehalose research.

Characterization and regulation of the trehalose synthesis pathway and its importance in the pathogenicity of *Cryptococcus neoformans*

Mylonakis, Eleftherios, Rude, Thomas, Toffaletti, Dena, Cox, Gary M, Miller, Jackie L, Perfect, John R CORA (2006)

Abstract: The disaccharide trehalose has been found to play diverse roles, from energy source to stress protectant, and this sugar is found in organisms as diverse as bacteria, fungi, plants, and invertebrates but not in mammals. Recent studies in the pathobiology of *Cryptococcus neoformans* identified the presence of a functioning trehalose pathway during infection and suggested its importance for *C. neoformans* survival in the host. Therefore, in *C. neoformans* we created null mutants of the trehalose-6-phosphate (T6P) synthase (TPS1), trehalose-6-phosphate phosphatase (TPS2), and neutral trehalase (NTH1) genes. We found that both TPS1 and TPS2 are required for high-temperature (37 degrees C) growth and glycolysis but that the block at TPS2 results in the apparent toxic accumulation of T6P, which makes this enzyme a fungicidal target. Sorbitol suppresses the growth defect in the *tps1* and *tps2* mutants at 37 degrees C, which supports the hypothesis that these sugars (trehalose and sorbitol) act primarily as stress protectants for proteins and membranes during exposure to high temperatures in *C. neoformans*. The essential nature of this pathway for disease was confirmed when a *tps1* mutant strain was found to be avirulent in both rabbits and mice. Furthermore, in the system of the invertebrate *C. elegans*, in which high in vivo temperature is no longer an environmental factor, attenuation in virulence was still noted with the *tps1* mutant, and this supports the hypothesis that the trehalose pathway in *C. neoformans* is involved in more host survival mechanisms than simply high-temperature stresses and glycolysis. These studies in *C. neoformans* and previous studies in other pathogenic fungi support the view of the trehalose pathway as a selective fungicidal target for use in antifungal development.

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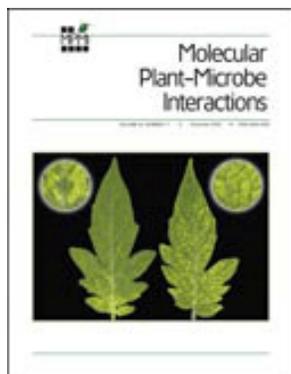
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Improvement of Drought Tolerance and Grain Yield in Common Bean by Overexpressing Trehalose-6-Phosphate Synthase in Rhizobia

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Improving stress tolerance and yield in crops are major goals for agriculture. Here, we show a new strategy to increase drought tolerance and yield in legumes by overexpressing trehalose-6-phosphate synthase in the symbiotic bacterium *Rhizobium etli*. *Phaseolus vulgaris* (common beans) plants inoculated with *R. etli* overexpressing trehalose-6-phosphate synthase gene had more nodules with increased nitrogenase activity and higher biomass compared with plants inoculated with wild-type *R. etli*. In

contrast, plants inoculated with an *R. etli* mutant in trehalose-6-phosphate synthase gene had fewer nodules and less nitrogenase activity and biomass. Three-week-old plants subjected to drought stress fully recovered whereas plants inoculated with a wild-type or mutant strain wilted and died. The yield of bean plants inoculated with *R. etli* overexpressing trehalose-6-phosphate synthase gene and grown with constant irrigation increased more than 50%. Macroarray analysis of 7,200 expressed sequence tags from nodules of plants inoculated with the strain overexpressing trehalose-6-phosphate synthase gene revealed upregulation of genes involved in stress tolerance and carbon and nitrogen metabolism, suggesting a signaling mechanism for trehalose. Thus, trehalose metabolism in rhizobia is key for signaling plant growth, yield, and adaptation to abiotic stress, and its manipulation has a major agronomical impact on leguminous plants.

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The following article and additional resources will supply you with a better understanding of neoformans discussed in a previous article about trehalose and neoformans.

A PEACH OF A PATHOGEN: CRYPTOCOCCUS NEOFORMANS

By Megan Simmer and David Secko

(August 2003)

When opportunity strikes you may just find *Cryptococcus neoformans* making the most of it. *Cryptococcus neoformans* is pathogen. *Cryptococcus neoformans* is also an opportunist. Over the past 10 years, infections of this pathogen have been on the rise, mostly striking individuals with compromised immune systems [1]. As an emergent disease it has taken advantage of the recent rise in the rates of immune-suppressing diseases, such as AIDS, as well as the use of drugs that suppress the immune system [1]. This interesting organism is increasingly being placed under a microscope to uncover how it is taking advantage of our immune systems.

A Shake of the Peach Tree

In 1894, a little bit of fermented peach juice introduced the world to an encapsulated yeast-like fungus that we now call *C. neoformans* [2] (see Figure 1). It was isolated from fruit juice by the Italian Sanfelice and shortly thereafter from the lesions of a woman [3], immediately revealing its potential as a human pathogen. Over the years, the organism

Sanfelice first isolated has been re-isolated and given all sorts of names, from *Saccharomyces hominis*, *Cryptococcus hominis* and *Torula histolytica*, all the way to *Debaryomyces hominis*. This created a little bit of confusion and the name was *C. neoformans* was finally decided upon to describe all the isolates [4].

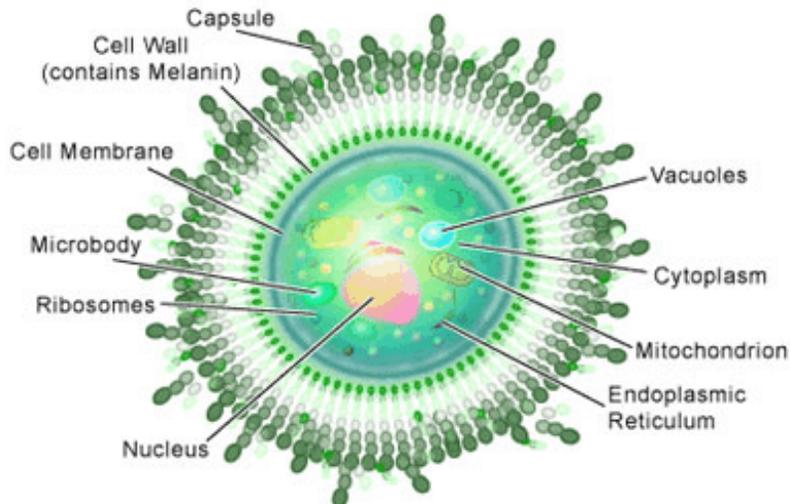
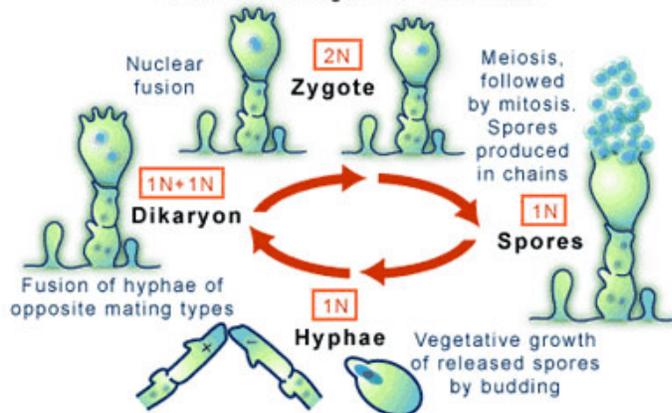


Figure 1
The life cycle of *C. neoformans*. The ploidy of the cells at the different stages is shown in red.



1. An idealized depiction of a *C. neoformans* cell. Note that melanin is found in the cell wall.

The Basic Biology of *C. neoformans*

If you examined *Cryptococcus neoformans* under a microscope you would see round or oval shaped yeast cells, which are often referred to as 'basidiomycete' yeast. Some

would have a scar on their surfaces where a daughter cell had budded off during reproduction. And if you happened to have some India ink lying around (a dye often used to stain cells) and applied it to them, you would observe a distinctive feature: a capsule made of polysaccharides (see Figure 1).

Basidiomycete fungi of this sort can produce spores, which occurs in another distinctive feature: a club-shaped structure called a 'basidium.' The production of spores is a result of the sexual reproduction of *C. neoformans* (see Figure 2), a process entirely different from its asexual reproduction by budding mentioned above.

Figure 2. The life cycle of C. neoformans. Notice how the growing cells produce hyphae that fuse, making a basidium. Spores will ultimately be produced on top of the basidium before being released into the environment.

Sexual reproduction in *C. neoformans* begins when two cells, each carrying one complement of their genetic information (a state in which they are called haploid), encounter one another and, as a result, fuse. The potential to fuse is regulated partly by the two cells being different mating types of which there are two flavors, “a” and “α”. The cycle of sexual reproduction, and thus of cell fusion, involves a series of mitotic cell divisions that produce thread-like extensions called hyphae (see Figure 2). At the end of the hypha the unique structure mentioned above, the basidium, is formed. It is the basidium that will bear the spores (sometimes called basidiospores) that will eventually form. For this to occur the two haploid nuclei residing in the basidium must fuse, through an event called karyogamy, to form one diploid nucleus. Meiosis and mitotic divisions will then go on to form spores. As these haploid spores are released to produce more cells the life cycle of *C. neoformans* will continue.

The Four Varieties

Four different types of *C. neoformans* occur throughout the world. Each type differs slightly in its global distribution and pattern of virulence. They are traditionally divided based on serotyping into variations A to D [5,6]. Two of the four types (B & C) are called *C. neoformans* var. **gattii**, while the others are known as *C. neoformans* var. **grubii** (A) and *C. neoformans* var. **neoformans** (D). It is the variations *grubii* and *neoformans* that are most commonly associated with disease.

In the northern hemisphere the most common forms of *C. neoformans* are var. *grubii* and var. *neoformans*, which are both most commonly found in pigeon poop [1]. Of the two varieties, it is *C. neoformans* var. *grubii* that makes up the vast majority of clinical strains isolated from infected individuals, while var. *neoformans* is not normally pathogenic. People who suffer from AIDS or who are receiving immunosuppressive drugs after an organ transplant are at risk of contracting **cryptococcosis**, the disease for which a *C. neoformans* infection is the cause [1].

In Australia and other sub-tropical areas of the southern hemisphere the most common form of *C. neoformans* is var. *gattii*, making it ecologically and epidemiologically different from *C. neoformans* var. *neoformans* and var. *grubii*. It is commonly found in association with eucalyptus trees and infections of var. *gattii* are restricted to lung infections. Interestingly, these infections occur in the immunocompetent population, with an rate of infection is eight per million per year in Australia. Table 1 summarizes the differences between *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*.

Strain	Global Distribution	Immune Status of Host	Disease	Environmental Reservoir
<i>C. neoformans</i> var <i>neoformans</i>	Temperate climates	Immunocompromised	Meningitis	Pigeon guano
<i>C. neoformans</i> var <i>gatti</i>	Tropical climates; lately isolated in a temperate zone	Immunocompetent	Pneumonia	Red Gum Eucalyptus trees

Table 1. A summary of the differences between *C. neoformans* var. *neoformans* and *C. neoformans* var. *gatti*.

Pathogenic Qualities

Cryptococcus as a genus contains approximately 37 species and out of all of them only *C. neoformans* is pathogenic [4]. This has made it not only a biological curiosity, but also a medical curiosity—especially since there has been a jump in the global rate of *C. neoformans* infections in the 1980s [1].

It is the occasional inhalation of the basidiospores that cause exposure to the pathogen, although these spores are normally dealt with by the immune system. But, when your immune system is not functioning properly those inhaled cryptococcal cells can reproduce and spread throughout your body. In such a case, cryptococcosis will result; commonly causing meningitis, which is an inflammation of the membranes surrounding the central nervous system (the brain and spinal cord). The incidence of cryptococcal meningitis being about ten per million per year, usually located in urban centers. Cryptococcosis has also been found to cause pneumonia and skin lesions.

The rise in *C. neoformans* infection is commonly linked to a global increase in the numbers of immunocompromised individuals, mostly closely associated with the AIDS epidemic [7]. The incidence of HIV-infected patients that develop cryptococcosis is around 10-15% in the Western Countries, but the effect of *C. neoformans* on AIDS is crystallized in Zimbabwe where the number jumps to 88% [8].

A Changing Picture of where *C. neoformans* is found

A traditional picture of the distribution of *C. neoformans* (see Table 1) and the incidence of cryptococcosis is beginning to change. Case in point, an outbreak of *C. neoformans* var. *gatti* infections on British Columbia's Vancouver Island [9]. A surprise to everyone, since this is the type of *Cryptococcus* is normally restricted to Australia. In 2001, British Columbia also saw an increase in the cases of cryptococcosis in pets, accompanied by a concomitant increase in the number of human cases.

The British Columbia Centre for Disease Control (BCCDC) launched an investigation into the disease. The detailed analysis that followed took a close look at medical records, undertook clinical reviews of all confirmed cases, environmental sampling, the identification of the pathogenic strains by genetic fingerprint analysis, and geographical mapping of the incidence of the disease.

As the results came in, it was found that since 1999 there have been over 40 incidences of cryptococcosis on Vancouver Island—all caused by *C. neoformans* var. *gatti*. The incidence of *C. neoformans* var. *gatti*, both in clinical and environmental samples, was found to be the highest on the central east coast of Vancouver Island. Visiting Rath Trevor Beach, a popular park in the area, turned out to be a significant risk factor for acquiring cryptococcosis. This was of interest since *C. neoformans* var. *gatti* is commonly found in association with Douglas fir, Garry Oak, Arbutus and Cedar trees. Activities like branch clean-up and chopping wood were thus found to be protective against cryptococcal illness. In 2001 the infection rate has peaked at 20 cases per million per year.

The BCCDC analysis has answered many questions about the outbreak of cryptococcal illness on Vancouver Island, but others remain. How did *C. neoformans* var. *gatti* end up on Vancouver Island? The fungus could have “hitch-hiked” here via infected trees imported from Australia or just naturally expanded its ecological niche due to climate changes. And more importantly, what has changed to increase the virulence of this fungus in comparison to its Australia counterpart?

Researching the Virulence of *C. neoformans*

Researchers have been working to determine how *C. neoformans* causes disease in humans and the bulk of this effort has been directed at understanding its virulence factors. Several have been identified and two of the most heavily studied are the secretion of a polysaccharide capsule and melanin synthesis [1].

Polysaccharide capsule—In response to low levels of iron in its environment and physiological levels of CO₂, *C. neoformans* can synthesize a thick polysaccharide capsule. The capsule interferes with the action of special immune system cells called macrophages that devour invading cells, thus protecting the *C. neoformans* cells from the immune system. Additionally, if macrophages do manage to engulf the cryptococci the capsule allows it survive and reproduce inside of the macrophage.

Melanin—This dark pigment is produced by the polymerization of compounds called polyphenols. It is a compound similar to the one that makes moles and freckles dark brown. For *Cryptococcus*, melanin offers protection from a process called oxidative killing. In host immune cells like macrophages, the killing of foreign cells is accomplished by exposing the engulfed foreign cells to highly reactive molecules called free radicals, which will react with molecules like DNA and proteins. These uncontrolled reactions often prove fatal. Melanin serves to protect cryptococci cells by reacting with free radicals to neutralize them.

Each of these factors aids in the survival of *C. neoformans* within its host, allowing it to evade the immune system and thus cause disease. It is for this reason that they are labeled as virulence factors.

Filling in the Gaps

It was over a hundred years ago that *C. neoformans* was discovered and since that time it has been scrutinized as a pathogen. But with cryptococcosis emerging in the past twenty years as one of the most-life threatening diseases in immune-compromised individuals, our understanding of its biology and pathogenic qualities is in need of gap filling. The medical research machine has been accelerating its focus on *C. neoformans* and many new discoveries are springing free, certainly making it a topic to keep your eye on.

Additional Reading

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(*Art by Jane Wang – note that high res versions of these image files are available [here](#)*)

<http://www.scq.ubc.ca/a-peach-of-a-pathogen-cryptococcus-neoformans/>

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