# The Truth About Trehalose
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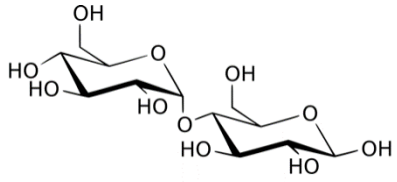
## Introduction

Trehalose is a naturally occurring disaccharide that is found in foods such as mushrooms, shellfish, seaweed (algae) and products made from brewer's yeast (e.g., beer and breads). Because of its stability and functional properties as well as recent innovations in manufacturing, trehalose has become pervasive in sweet and savory foods, e.g., baked goods, frozen desserts, jams and jellies, rice bowls, salads, meat and fish dishes. Paralleling the increased production and widespread utilization of trehalose has been an increase in the incidence of hospital infections with an antibiotic resistant strain of intestinal bacteria—*Clostridium difficile* (*C. difficile*). Researchers from Baylor College of Medicine and the University of Oregon have tried to link these two seemingly arbitrary occurrences together in a series of epidemiological and microbiological studies, which has led to media reports and cautionary advice to avoid dietary trehalose (Collins *et al.* 2018 & Collins *et al.* 2019). But are these warnings warranted?

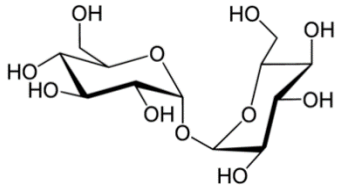
## Trehalose: Quite Simply, a Simple Sugar

Trehalose is a disaccharide comprised of two glucose molecules bonded together. What differentiates trehalose from the more common disaccharide maltose (also consisting of two glucose molecules bonded together) is the bond that connects the glucose molecules. That is, trehalose contains "an α,α-1,1 glycosidic bond," while maltose contains "an α-1,4 glycosidic bond." Additional factors that differentiate the two glucose containing disaccharides include the fact that maltose is a reducing sugar, whereas trehalose is not, and trehalose is significantly more "stable" than maltose under high temperatures and acidic pH (Richards *et al.*2002).

**The structures of maltose and trehalose are shown below. Compare and contrast these carbohydrates.**




**Maltose**




**Trehalose**

The digestion and absorption of trehalose in humans is similar to that of other disaccharides. Trehalase, an enzyme produced and secreted from the small intestine brush border, cleaves the glycosidic bond, releasing two molecules of glucose which are subsequently actively absorbed by the intestinal mucosal cell via sodium glucose transporter 1 (SGLT 1) (Elbein 1974 & Richards *et al.* 2002).

Trehalose occurs naturally in small amounts in a variety of foods most notably mushrooms, honey, lobster, shrimp, certain seaweeds (algae), wine, beer, bread and other foods produced by using baker's or brewer's yeast (Richards *et al*. 2002). It can also be manufactured from a variety of plant-based starches using enzymatic processing procedures (Maruta *et al.* 1995). Because it is heat-stable, hydrophilic and resistant to acidic hydrolysis, trehalose has been used a texturizer, stabilizer and occasionally a sweetener in dried foods, frozen foods (e.g., ice cream), nutrition bars, fruit fillings and

jams, instant noodles and rice, baked goods and fruit juices (Richards *et al.* 2002). Trehalose gained "*generally recognized as safe*" (GRAS) status by the U.S. Food and Drug Administration in 2000 and was approved for use in foods in Europe in 2001 (Richards *et al.* 2002).

**The "Smoking Gun?"**

In January of 2018, a paper was published in the journal *Nature* linking trehalose to a particularly "virulent" (i.e., infectious) strain of *C. difficile* (Collins *et al.* 2018). Specifically, the paper summarized a series of *in vitro* and animal experiments showing that while the virulent strain can thrive on a number of different carbon sources (e.g., simple sugars, sugar alcohols, amino acids and some small proteins), it is able to grow robustly on low doses of trehalose. According to the authors of the *Nature* paper, this finding was the "smoking gun"—the conclusive evidence for the microbiological role of trehalose in the growth and proliferation of the infectious strain of *C. difficile* (Abbasi 2018). This data, combined with observations that increased production and utilization of trehalose in the food supply coincided with the rise of infections caused by the virulent strains of *C. difficile*, led to speculation that trehalose caused the epidemic emergence of the virulent strain of *C. difficile* (Collins *et al.* 2019).

**Does Trehalose Cause *C. difficile*?**

The short answer is "no."

The long answer requires a slightly more in-depth evaluation of the existing research on trehalose, beginning with the *Nature* paper.

The *Nature* paper is not a single study, but rather a compilation of summaries of individual experiments each conducted by one or more of the authors listed on the paper. Several experiments described in the paper simply examined the genetic variants of the different strains of *C. difficile*, including the most virulent strains, to characterize the microbiology and physiology. Another experiment tested several carbon sources (i.e., various carbohydrates and amino acids) to determine if they supported the growth of a particular virulent strain of *C. difficile in vitro* (that is, in a petri dish). Interestingly, the findings indicated that several carbohydrates and amino acids (not just trehalose) caused a 1.5-fold increase in the virulent strains. Nonetheless, the researchers chose only to focus on trehalose, allegedly because unlike some of the other carbon sources studied, trehalose supported growth even at relatively low concentrations. The experiment that probably garnered the most media attention involved feeding mice either trehalose or water. The results showed that the trehalose-fed mice had an increase in the growth of the resistant strains of *C. difficile*. These results are not surprising. Of course, we would expect to see greater growth of *C. difficile* on a carbohydrate source compared with water. A better, more equitable comparison would have been another sugar (or more than one sugar), particularly one that has been shown in previous experiments to stimulate the growth of *C. difficile*.

One other final experiment in the *Nature* paper that is worth noting examined the metabolism of trehalose in the intestines of three human subjects consuming their "normal diets" (which strangely were never described in the paper). The results of this final experiment showed that in two of the three subjects, there was an increased expression of the "*treA* gene," which is required to metabolize trehalose. No statistics were run on this "sample" likely because it was too small and there really wasn't anything to compare or calculate. In addition, the study did not show that eating a "normal diet" containing trehalose increased levels of *C. difficile* bacteria in the gut, nor that it caused symptoms; only that it increased the expression of a gene that metabolizes trehalose.

Thus, the data reported in this paper does not show that trehalose causes the growth and proliferation of resistant strains of *C. difficile* in humans. It does show that the resistant strains of *C. difficile* can grow *in vitro* on low doses of trehalose, but they metabolize and flourish on several other carbon sources as well (and, as noted, none were directly compared to trehalose). Moreover, growth *in vitro* in a laboratory setting does not necessarily generalize to a human gut. The "feeding" experiment was conducted in a mouse model (which may or may not translate to a human model) and did not include a carbohydrate comparison. Finally, the only *in vivo* experiment described in the *Nature* paper included just three subjects and measured changes in the expression of a gene that metabolizes trehalose (not development or proliferation of *C. difficile*).

**Disconnecting the Dots**

More recent research not only contradicts findings from the *Nature* paper, but refutes a causal connection between trehalose consumption and *C. difficile* infections.

Eyre and colleagues (Eyre *et al.* 2019) examined the potential for trehalose metabolism variants to confer a select advantage for the virulent strains of *C. difficile* by evaluating how common these variants are within the total genetic diversity of clinical *C. difficile* using previously sequenced isolates. In addition, the researchers compared the effects of trehalose, glucose or saline supplementation on *C. difficile* metabolism using a validated and clinically reflective gut model of *C difficile*. The results indicated that trehalose metabolism/utilization variants are quite common among the population and supplementation of trehalose did not result in increased levels of *C. difficile* or its spores compared with glucose or saline supplementation. Quite the contrary, supplementation with trehalose actually reduced toxin detection to undetectable levels.

In a recently published case-controlled study (Saund *et al.* 2020) evaluated the potential contribution of trehalose consumption to clinical outcomes in 1144 hospitalized patients with *C. difficile*. After controlling for all clinical factors independently associated with risk for severe infection outcome, the authors found no significant association between the presence of trehalose utilization variants in infectious *C. difficile* strains and the development of severe infection outcomes.

Notably in both studies, trehalose metabolism/utilization variants were more widespread than previously thought, suggesting that the ability to utilize low concentrations of trehalose was acquired prior to the recent increase in trehalose production and utilization (Eyre *et al.* 2019 & Saund *et al.* 2020).

**Association ≠ Causation**

While the oft-cited association between trehalose production/utilization and *C. difficile* proliferation may seem compelling, other epidemiological observations suggest that factors besides trehalose consumption may be contributing to the increase in virulent strains of *C. difficile* (Buckley e*t al.* 2021). For example, in Canada, outbreaks of the virulent strains of *C. difficile* occurred some three years before the approval and expanded use trehalose. In addition, data from the U.S. Center for Disease Control (CDC) indicates that at least one of the virulent strains of *C. difficile* is actually declining, despite the fact that no active measures have been taken to reduce trehalose production or consumption (Abasi 2018). Finally, and probably most convincingly, research evaluating the extent to which imports of synthetic trehalose altered total dietary trehalose intake during the rise of the virulent strains of *C. difficile* in

Europe, the U.S. and Canada and showed that the increase in trehalose imports postdated the start of *C. difficile* epidemics in these countries by at least five years (Eyre *et al.* 2019).

**The Bottom Line**

Trehalose is a naturally occurring disaccharide that has been consumed by humans for centuries and used in food production for decades—long before the epidemic rise of virulent strains of *C. difficile*. While *in vitro* data shows that virulent strains of *C. difficile* proliferate when exposed to low levels of trehalose, *in vivo* data in humans using appropriate comparisons and controls is generally lacking and the few existing studies have failed to confirm these results. Finally, the majority of existing epidemiological research does not support a valid association between trehalose production/usage and the emergence of resistant strains of *C. difficile*. Thus, the preponderance of evidence indicates eating foods containing trehalose is safe and will not significantly increase the risk of developing resistant strains of *C. difficile* (Buckely *et al*. 2021).

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