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An Additive Sugar Helps the *C. diff* Go Round

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Outbreaks of hypervirulent strains of *Clostridium difficile* began to be reported in healthcare facilities worldwide around 20 years ago. Concurrently, trehalose became a common additive used by the global food industry. A new study provides evidence that these two observations are a linked phenomenon (Collins et al., 2018).

Food consumption is the predominant source of nutrients for bacterial communities residing within the mammalian intestinal tract. Changes in diet result in altered community structure as species must adapt to new energy sources or be eradicated from the competitive intestinal niche (David et al., 2014). Numerous associations between the composition of intestinal microbial communities and human physiology have been identified (Cho and Blaser, 2012). Therefore, diet represents a key lever in shaping microbial community composition and, in turn, health and disease. The recent report from Britton and colleagues (Collins et al., 2018) identifies a dietary sugar, trehalose, that supports the growth of the pathogenic intestinal bacteria *Clostridium difficile.* This work provides a clear, practical example of diet shaping host health via intestinal bacteria.

C. difficile can infect the large intestine following disruption, often via antibiotic treatment, of the indigenous microbiota, causing debilitating, sometimes fatal colitis (Abt et al., 2016). Over the past quarter of a century *C. difficile* has become one of the most pervasive and challenging nosocomial pathogens, with outbreaks of hypervirulent strains occurring in hospitals throughout the world. *C. difficile* strains belonging to the PCR-ribotype 027 and 078 lineage are prominent sources for these recent outbreaks (Martin et al., 2016). The etiological forces driving ribotype 027 and 078 to emerge as worldwide epidemic strains are the source of intense study. Both lineages have been found to be increased toxin producers (Goorhuis et al., 2008; Warny et al., 2005), while resistance to fluoroquinolones has also been linked to epidemic outbreaks (Martin et al., 2016).

Britton and colleagues interrogated this question by comparing the competitive fitness of epidemic strains (ribotype 027 and 078) against non-epidemic strains. Previous work by this group demonstrated that strains from the ribotype 027 lineage exhibited increased persistence in the context of a complex microbiota compared to non-epidemic isolates (Robinson et al., 2014). Their current work identifies the molecular mechanisms behind the competitive fitness advantage of both ribotype 027 and 087 lineages.

A carbon utilization screen comparing the growth of epidemic (ribotype 027) versus non-epidemic (ribotype 012) *C. difficile* strains identified the disaccharide trehalose as a carbon source that preferentially supported growth of the ribotype 027 strain. Subsequent screening of 21 different strains in minimal media with trehalose as the sole carbon source revealed that only strains from ribotype 027 or 078 lineages exhibited enhanced growth at low concentrations of trehalose.

The authors next identified the molecular mechanisms that supported trehalose metabolism in these two ribotype lineages (Figure 1). Whole-genome analysis of multiple *C. difficile* strains revealed a putative phosphotrehalase enzyme (TreA), which metabolizes trehalose into glucose, and a corresponding transcriptional repressor (TreR) in the genome of both epidemic and non-epidemic *C. difficile* strains. However, sequence alignment analysis comparing the trehalose operon between more than 1,000 sequenced *C. difficile* strains revealed a single nucleotide polymorphism (SNP) in all ribotype 027 strains analyzed. This SNP resulted in a leucine-to-isoleucine switch at a site on the TreR protein near the trehalose binding pocket. This mutation could inactivate TreR and enable increased sensitivity to trehalose. Indeed, the ribotype 027 strain was observed to turn on the *treA* gene at trehalose concentrations 500-fold lower than

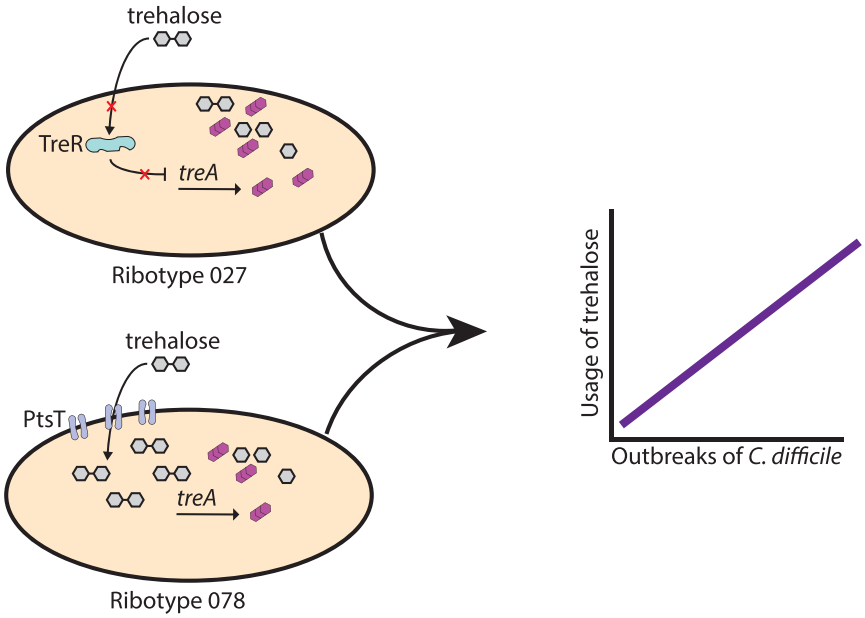

Figure 1. Two Independent Trehalose Utilization Mechanisms in Distinct Epidemic Strains of *C. difficile*

Collins et al. (2018) report two unique mechanisms of trehalose utilization in the ribotype 027 and 078 lineage of *C. difficile*. Ribotype 027 strains have a mutation in the trehalose transcriptional repressor (TreR), and ribotype 087 strains encode for an additional trehalose transporter (PstT). The emergence of these ribotypes as epidemic strains of *C. difficile* has coincided with increased usage of trehalose as a food additive.

a non-epidemic strain. Further, mutant strains lacking the treA gene (Δ*treA*) lose the capacity to grow in the presence of trehalose, demonstrating the necessary role of the *treA* operon in trehalose utilization. When wild-type and Δ*treA* mutants were administered to mice supplemented with trehalose in their diet, Collins et al. (2018) observed that infection with the wild-type strain resulted in increased mortality compared to Δ*treA*-infected mice. These findings demonstrate that trehalose utilization by *C. difficile* confers increased disease severity.

Fascinatingly, whole-genome analysis revealed an entirely independent molecular basis in the ribotype 078 lineage for trehalose utilization. A four-gene insertion encoding a second phosphotrehalose enzyme (TreA2) and putative trehalose transporter (PtsT) was identified in all ribotype 078 strains. Deletion of the *pstT* gene in ribotype 078 strains abrogated the mutant's capacity to grow at low trehalose concentration levels. Further, expression of the *ptsT* gene by an inducible promoter in a non-epidemic strain was sufficient for growth at a low trehalose concentration. Thus, the authors identified two evolutionary independent mechanisms that drive trehalose sensitivity. In the ribotype 027 lineage, a mutation in the TreR repressor enables elevated expression of the phophotrehalase enzyme, while in the ribotype 078 lineage a gene insertion provides an additional means of trehalose uptake (Figure 1).

Trehalose is naturally found in food products such as mushrooms, honey, and baker's yeast (Richards et al., 2002). Endogenous trehalase expressed on the brush border of the small intestine in most mammals, including humans, breaks down ingested trehalose into glucose for absorption. This process was historically sufficient to deprive large-intestine-residing microbes, such as *C. difficile*, of access to trehalose as a carbon source. However, large-scale commercial availability of trehalose rapidly increased in the late 1990s with the development of a method to derive trehalose from starch, which rendered trehalose an economically viable option for the food industry (Maruta et al., 1995). The long-term stability and resistance to heat and acid has made trehalose a popular additive in the global food supply chain and perhaps overwhelmed the body's natural capacity to breakdown and absorb trehalose byproducts. Collins et al. (2018) sought to determine if physiologic amounts of trehalose could pass through the small intestine without being absorbed. Using *treA* gene expression as a readout for the presence of trehalose, the cecal content of mice administered a concentrated dose of trehalose or the ileal effluent from human volunteers was incubated with a ribotype 027 strain. Induction of the *treA* gene following incubation was observed in both cases, supporting the conclusion that ingested trehalose can reach the large intestine to be consumed by bacteria as an energy source.

Increased trehalose consumption coinciding with multiple outbreaks of *C. difficile* derived from strains that are predisposed to benefit from trehalose availability is an observation that has provocative connotations regarding food regulation. Importantly, the authors note that the molecular machinery that increases sensitivity to trehalose in ribotype 027 and 078 lineages was present prior to the epidemic outbreaks of the last 20 years. It is unclear what the evolutionary pressures were that hardwired trehalose utilization in the genomes of these distantly related ribotypes. Are there other intestinal bacteria species that possess trehalose utilization capabilities? And if so, have these species bloomed in prevalence over the past two decades? Britton and colleagues (Collins et al., 2018) report that ribotypes phylogenetically related to the 027 and 078 lineages possess the same machinery to utilize trehalose. Why some of these lineages are linked to hypervirulent outbreaks while others are not remains to be determined. Addressing these unresolved issues will yield valuable insights into the epidemiology of *C. difficile*-associated disease and could further instruct our understanding of how diet shapes intestinal bacterial communities.

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