Probiotics: A review of potential alternative & effective treatments against *C. difficile*

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Abstract

Clostridioides difficile is a bacterial pathogen that generally affects people that are immunocompromised and/or on heavy courses of antibiotics (CDC, 2022). Current treatments for the pathogen include using fecal transplants and/or probiotics (*Fecal Transplant* -- Hopkins medicine, 2022) – the latter of which can be consumed through everyday foods or through supplements (CDC, 2022). Probiotics are live microbes that are thought to supplement and/or mimic the beneficial attributes of our gut microbiome, a collection of organisms that are needed for performing essential functions in the body such as producing certain vitamins or, as seen in the case of *C. diff.,* staving off pathogens (Cleveland Clinic, 2022). This review article will discuss what probiotics are & how they can be obtained, how they are thought to provide health benefits, and how probiotics are capable of outcompeting pathogens such as *C. diff.* (Buonomo & Petri, 2016).

Introduction

Clostridioides difficile (C. diff.) is a bacterial opportunistic pathogen estimated to cause almost half a million infections in the United States each year (CDC, 2022). It generally infects people after taking certain antibiotics and/or if they are immunocompromised (CDC, 2022). While common treatment options to fight this infection involve prescribing alternative antibiotics, a newer approach involves using live microbes to outcompete the pathogen. Probiotics are one example of using live microbes and, depending on what species are ingested, could provide various health benefits (such as restoring the gut balance). This article will focus on explaining: 1) what probiotics are; 2) how probiotics can be obtained; 3) how probiotics provide health benefits; and lastly 4) what mechanisms they use to fight off infections such as *C. diff*.

What are probiotics?

The human body is covered by densely populated communities of microorganisms, the majority of which live in the gastrointestinal tract (Cleveland Clinic, 2020). These microbes – which include bacteria and fungi – are genetically & metabolically diverse and provide multiple health benefits for the body including: breaking down dietary fiber and nutrients, preventing pathogens from colonizing & growing, producing certain vitamins, and even modifying certain neuronal hormones. In terms of numbers, the human body is thought to have almost the same number of bacterial cells as human cells; while absolute quantification is challenging, the gut is thought to have roughly 100 trillion microbial cells (Guinane & Cotter, 2013).

The term probiotic, more specifically, refers to live microbes that are thought to provide similar benefits as our “microbiota” when ingested. Depending on the source, they can consist of many different microorganisms including specific types of bacteria and fungi such as *Bifidobacterium, Lactobacillus, Lactococcus,* and *Saccharomyces boulardii* (*Office of Dietary Supplements - Probiotics*, 2018).

While research into probiotics is still new, it is accepted that certain species are capable of providing certain unique functions based on their genome. *Bifidobacteria*, for example, not only help with “staving bacterial pathogens”, but also encode genes that can aid with the conversion of dietary fibers into beneficial molecules (Duggal, 2017). They do this by metabolizing and fermenting the fiber to create short-chain fatty acids (SCFAs) which serve as an energy source for cells lining the colon wall. These molecules can additionally modulate and dampen the immune system, giving them a major role in maintaining gut health (Mei et al., 2022). For example, butyrate – an SCFA produced by the metabolization and fermentation of fiber – is known to promote anti-inflammatory effects in certain immune cells (Muhammad Anshory et al., 2023).

Beyond providing energy and molecules that can modulate the immune system, probiotics can (depending on the species) produce other useful molecules for our body, including vitamins. *Lactobacillus*, for example, not only makes lactic acid to create an inhospitable environment for bad bacteria (described later) but also produces several strains of B vitamins like "folates, riboflavin and vitamin B(12)" (Jean Guy LeBlanc et al., 2011). These vitamins are not normally produced by our human cells.

How can we obtain probiotics?

There are many ways that we can obtain probiotics, one of these being through foods that are eaten on a daily basis. Arguably the easiest source can come from fermented foods like some types of cheeses, breads, yogurt, kimchi, and kefir. These foods generally have high abundances of certain types of microbes that can ferment sugars; for example, yogurts contain a vast amount of *Lactobacillus acidophilus*, whereas sourdough bread can harbor *Candida milleri* and *Lactobacillus sanfranciscensis* (*How to Get More Probiotics - Harvard Health*, 2020). These microbes often produce acids while breaking down the sugars and starches present, giving the food a distinctive "sour" taste.

Another way is over-the-counter supplements that can be obtained at many retail stores and pharmacies. For a given supplement, the manufacturer will normally have a "supplemental facts" section on the box that lists the variety of microbes allocated in each pill and the amount of total live microbes that are present. That said, purchasing probiotic supplements should come with careful considerations; in terms of the contents found in a given pill, manufacturers could insert an arbitrary amount of live microbes at undisclosed ratios. Additionally, as the field is still relatively new, it's unclear what benefits a given microbe will provide and/or whether the microbe will reach the correct place in the intestine. Lastly, if a probiotic works for one person, there is no guarantee it will work for another individual as each person has their own unique microbiome.

On the regulatory front, there are currently no FDA-approved probiotics. Probiotics are typically sold as "dietary supplements and do not require FDA approval unless they make health claims" (FDA Center, 2022).

How does *C. difficile* cause disease?

As described earlier, our gut microbiome is home to a densely populated community of microbes that help protect us from pathogens. If a large disturbance to that community occurs, such as through the use of antibiotics, opportunistic infections could lead to unwanted side effects and, in some cases, severe disease. A leading example of this involves *Clostridioides difficile (C. diff)*, a bacterial "pathobiont" that can cause diarrhea and colitis in humans (CDC, 2022). Interestingly, many of us naturally live with *C. diff.* and have no side effects if we have a "healthy" and diverse microbiome. However, the bacteria can thrive and cause disease in low-diversity microbiomes – often seen in individuals who are immunocompromised or are taking heavy courses of antibiotics (*What Is an Opportunistic Infection? | NIH*, 2021).

Epidemiologically, *C. diff.* is a prime cause of hospital-acquired diarrhea in the United States, with more than 250,000 cases every year (Voth & Ballard, 2005).

Mechanistically, it's thought *C. diff.* mainly affects the body by releasing toxins that damage internal tissues (*C. Difficile Infection - Symptoms and Causes*, 2021). These toxins, called TcdA and TcdB, attack your cells and cause lasting inflammation and damage to your intestinal lining (Voth & Ballard, 2005). This, in turn, can lead to diarrhea and colitis. These toxins may be released in response to environmental signals; though understudied, two leading hypotheses as to why *C. diff.* releases toxins include the restriction of certain types of nutrients & metabolites (such as biotin) and/or stress on the pathogen caused by antibiotics (Voth & Ballard, 2005).

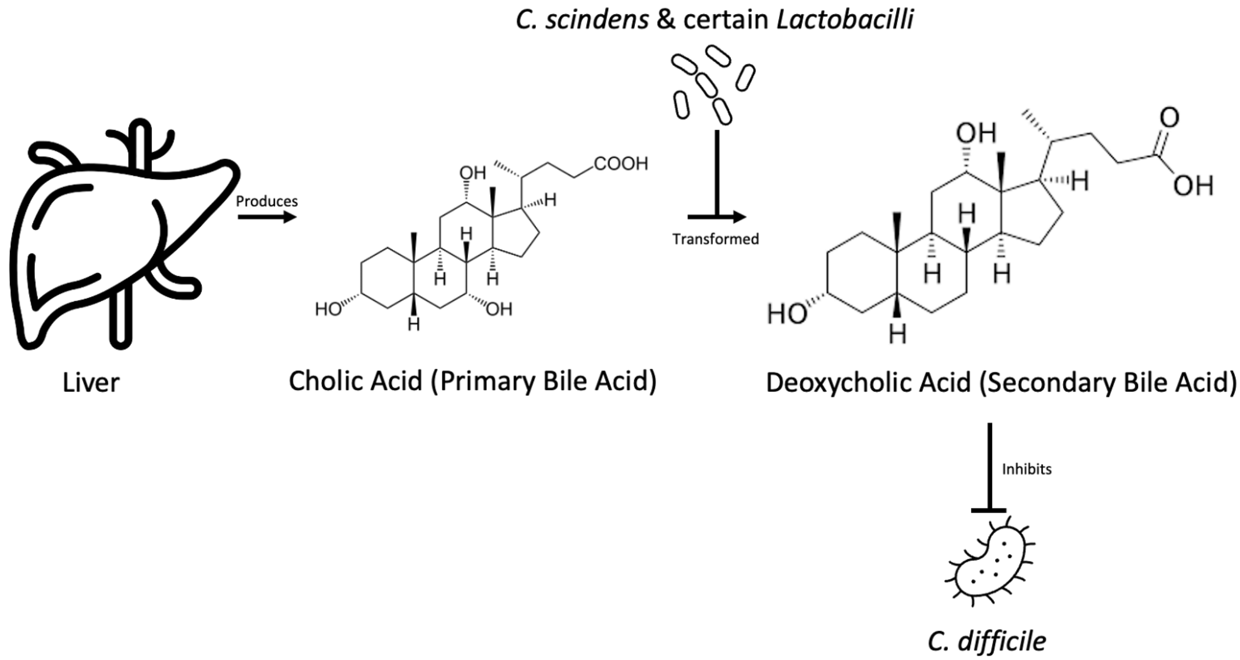
In terms of treatment, patients are often given antibiotics specific against *C. diff.* or can undergo what is known as a fecal transplant – a situation where stools from healthy donors are added to a patient's colon to restore the microbiome's protective function (*Fecal Transplant* -- Hopkins medicine, 2022).

What mechanisms do probiotics provide to help fight infections such as *C diff.?*

While *C. diff.* is capable of causing serious infections in humans, the primary (and arguably most effective) treatment option involves restoring a patient's microbiome to a healthier, more diverse state (CDC, 2022). Fecal transplants, a process mentioned above, tries to quickly and efficiently accomplish this by simply reintroducing hundreds of different species of microbes directly to the gut environment. Since these microbes encode hundreds of unique genes, these microbes can (in theory) both physically and genetically outcompete *C. diff.* by creating an environment that isn't suitable for it (Buonomo & Petri, 2016).

Fecal transplants, however, have two major issues: 1) the mechanisms in which a more diverse microbiome can outcompete the pathogen are unclear and understudied; and 2) there are major concerns about the safety of using this process (*Fecal Transplant* -- Hopkins medicine, 2022). While donor stools are screened for the presence of certain pathogens, there is always a risk of secondary infections.

An alternative treatment that is currently being explored is providing patients with certain probiotics & microbes that can directly attack the pathogen or generate molecules that can negatively impact it. An example for *C. diff.* involves "secondary bile acids" (Mills et al., 2018). The liver produces bile acids that are secreted into the intestine to help break down fats that are ingested. Select microbes in the intestine are able to convert & metabolize these "primary" bile acids to "secondary" bile acids. Research has shown secondary bile acids, such as deoxycholic acid and lithocholic acid, can negatively impact *C. diff.* growth, limiting its ability to produce toxins (Tam et al., 2020).



Schematic on how transformed bile acids can be used to inhibit pathogens
Our bodies naturally produce certain types of bile acids (such as cholic acid) to help digest lipids and fats that are ingested. Certain microbes in the gastrointestinal tract are capable of metabolizing these “primary” bile acids, transforming them into “secondary” bile acids (such as deoxycholic acid). These transformed molecules can negatively impact certain pathogens, including *C. difficile.*

Beyond *C. difficile,* there are multiple other mechanisms that probiotics provide to defend against pathogens. The bacteria *Lactococcus lactis,* for example, is found in many cheeses and milk and is usually taken as a therapy for the gastrointestinal system or just for the prevention of disease or to repel already present pathogens (Mills et al., 2011). Beyond converting proteins in milk to flavorful compounds, the species can produce lactic acid and other SCFAs. As described earlier, these molecules can directly inhibit the growth of pathogens and/or spur the immune system to be more or less reactive (Mills et al., 2011).

Conclusion

Microbes play a critical role in nature. In terms of the human body, they can help break down & metabolize foods and prevent certain diseases & infections. Probiotics are live microbes that, if consumed, are thought to improve health by restoring and/or supplementing the gut microbiome found in a given person. While research into probiotics is limited with not a lot of information compared to many other fields, there are multiple pieces of evidence in the literature to suggest that probiotics can be beneficial, such as helping to fight off infections such as *C. difficile.* They are additionally easy to obtain – consuming certain fermented foods, for example, can introduce beneficial microbes to the gut – and may be useful alternatives to antibiotics. While antibiotics can be an effective treatment against bacterial infections, they can consequently destroy the balance in the microbiome allowing for certain pathogens to bloom. Using probiotics to combat (or even prevent this in the first place) may be beneficial with limited side effects. Any decision to use probiotics, however, should be guided by consultations with experts and medical professionals.

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