Lab Report

Utilization of chitinase for digestion of indigestible fibers like chitin to alleviate symptoms and inflammation in irritable bowel disease *

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Reviewed on 4 May 2024; Accepted on 10 June 2024; Published on 26 October 2024

Irritable Bowel Diseases (IBD), such as Irritable Bowel Syndrome (IBS), are chronic health conditions that cause disruptive and challenging symptoms. Chitin, a type of indigestible fiber, often exacerbates IBD symptoms. Studies suggest that the ability to digest chitin could lessen IBD symptoms. This experiment will utilize chitinase to break down chitin, thus alleviating symptoms of IBD. This project aims to generate a probiotic bacterium that is able to digest chitin.

Keywords: Chitin, chitinase, irritable bowel disease (IBD), inflammation, symptoms, bacteria

Irritable Bowel Disease (IBD) is a common gastrointestinal disease that affects millions of people worldwide, often causing gastric discomfort and inflammation (Bollyky et al., 2018; Du et al., 2020; Fischna et al., 2021; Patel & Shackelford, 2022). Chitin is a type of indigestible fiber that can exacerbate IBD symptoms. Additionally, chitin has been shown to benefit harmful bacteria, increasing the concentration of harmful microbes. Over time, the enlarged population of these bacteria directly worsens IBD symptoms, as well as causes systemic autoimmunity (Bollyky et al., 2018; Du et al., 2020; Fischna et al., 2021). Recent studies suggest that chitinase, which breaks down chitin, may have evolved for various protective functions associated with inflammatory pathologies such as IBD (Bollyky et al., 2018; Fichna et al., 2021). Chitinases directly diminish the unwanted presence of chitin, thus resulting in a decreased occupancy of harmful microbes in the gut microbiome. More beneficial microbes will surface in the absence of chitin and their adverse counterparts, relieving the symptoms of IBD and its other associated effects on the human body (Patel & Shackelford, 2022)

Introduction

Irritable bowel disease (IBD) is a common

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gastrointestinal disease that impacts the stomach and intestines, otherwise known as the gastrointestinal tract (Mayo Clinic, 2023). A study estimates that nearly 1 in 100 Americans have inflammatory bowel disease, and 56,000 new cases are diagnosed each year. An estimated 2.4 million Americans have some form of IBD; it is imperative to see that it is gradually increasing (Brooks, 2023). The cause of this condition is unknown. However, it has been related to various factors such as abdominal hypersensitivity, abnormal intestinal motility (diarrhea or constipation), gut dysbiosis (an imbalance of bacteria in the gut), genetics, and psychological factors. IBD is an umbrella term used to describe disorders that cause chronic inflammation of the digestive tract. Symptoms may include diarrhea, belly pain, nausea, fever, loss of appetite, fatigue, and rectal bleeding. The two most common forms of IBD are Crohn's disease and ulcerative colitis (UC). There are no cures (Brooks, 2023; Mayo Clinic, 2023).

Current treatment

The current and common treatments for IBD vary between antidiarrheal drugs, anticholinergics, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and anticonvulsants as represented in Table 2 (Bharadwaj, 2023).

Nutrition therapy

Even though the high prevalence of IBD pathophysiology is partly understood and multifactorial, many patients report symptoms that happen to be meal-related and certain ingested foods may cause a gastrointestinal response (Farrow, 2024). Because of this, patients tend to avoid or exclude certain foods to relieve their symptoms, which affects their nutritional quality. Considering IBS nutrition therapy options between fibers or hydration. Fibers may trigger symptoms for individuals, and for others, the need to increase fluid intake to support stool viscosity and transit if fiber is increased (Farrow, 2024).

Table 1. Medication List. The information here is from the CDC.

Medication type	Information	Examples
Antidiarrheal drugs	Relieves diarrhea	Loperamide, Cholestyramine, and Colestipol
Anticholinergics	Relieves spasms	Dicyclomine
Tricyclic antidepressants	Relieves depression and severe pain	Imipramine and Desipramine
SSRIs	Relieve depression, pain, and constipation	Fluoxetine and Paroxetine
Anticonvulsants	For pain and bloating	Pregabalin and Gabapentin

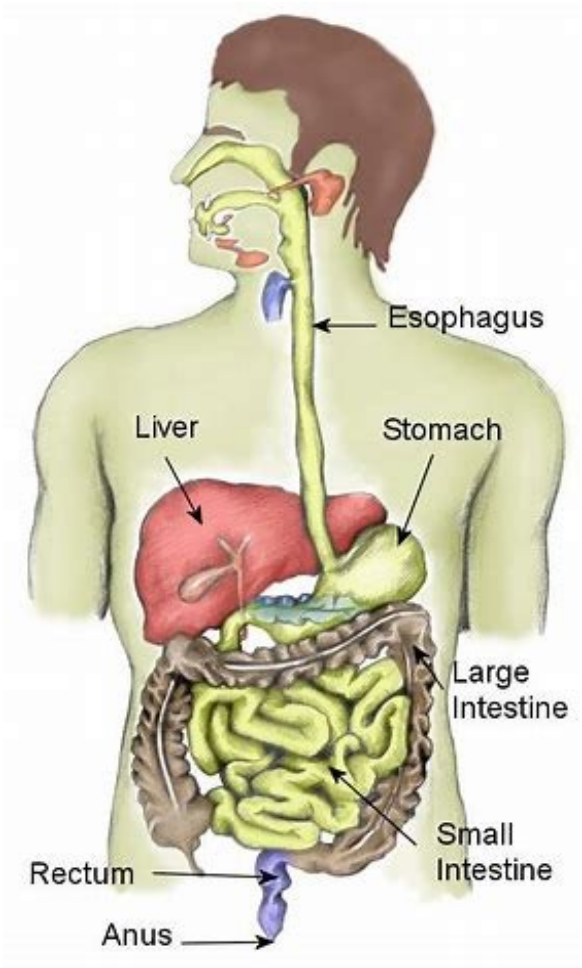

Figure 1. Human Digestive System. https://www.teachpe.com/anatomy-physiology/anatomy-of-the-spine

Probiotics

Probiotics help support gut health. Probiotics are supplements that contain microbes when taken in adequate amounts to provide benefits for health. It is believed that many people, not just those with IBD, have unbalanced gut bacteria. Recent studies indicate that the intestinal microbiota plays a role in initiating, maintaining, and

determining the characteristics and development of IBD (IBDrelief, n.d.; Mayo Clinic, 2023)). In addition, many people with IBD report that taking probiotics can help alleviate symptoms (IBDrelief, n.d.). However, studies into the effectiveness of probiotics on people with IBD are limited. There have been little to no studies into specific strains of bacteria and much fewer studies on testing probiotic products on IBD. In current research, specific probiotics may be helpful for people with UC. However, there is not much evidence that probiotics may be helpful for people with UC (IBDrelief, n.d.).

Chitin and the potential of chitinase

Chitin, a polysaccharide commonly found in cell walls, can potentially exacerbate IBD by triggering an immune response and leading to the activation of immune cells and the release of pro-inflammatory cytokines (Bollyky et al., 2018; Cruz et al., 2018). To combat this, chitinase, an enzyme that breaks down chitin, can be used as a probiotic to break down chitin into smaller monomers and thereby alleviate inflammation and symptoms of IBD (Bollyky et al., 2018; Fichna et al., 2021).

Materials and methods

To create a chitinase-producing system, we needed to insert a chitinase gene into a cloning vector—we chose the T7-P14 vector, as we could use the expression of its preexisting GFP protein as evidence of chitinase expression. We inserted the start codon of chitinase downstream of the T7 promoter, which ensures that chitinase is expressed alongside GFP, as the GFP gene precedes the T7 terminator.

Testing this system will involve inserting the cloning vector into a dish with the cell-free system and an inducer. The efficacy of our chitinase-producing cloning vector can be shown quantitatively by measuring the amount of light emitted from the GFP.Out of the options at our disposal, we decided that utilizing the BioBuilder lab *Ready, Set, Glow* kit would be most suited to our experiment. For one, it corroborates with the use of the T7-P14 vector, as this transcription device produces Green Fluorescent Protein alongside the chitinase protein. The kit is additionally a cell-free system, which allows us to evaluate the efficacy of the cloning vector without the complexity of a microorganism. Our priority was making sure the whole system itself functioned before moving on to incorporating it into a real-world application, and the *Ready, Set, Glow* kit provides the most efficient way of doing this. Most importantly, however, the kit would make it apparent if transcription and translation had occurred, given the amount of green fluorescence that is present; there would be no ambiguity whether translation and transcription took place. This in itself makes the procedure much more straightforward.

In the first trial, utilizing the BioBuilder lab *Ready, Set, Glow* kit and procedure, the cloning vector was tested with four groups: a positive control group, two negative control groups, and an experimental group. The positive control group included one sample of the provided TxTL Master Mix (20 µL), GFP DNA (5 µL), and Inducer DNA (1 µL). The positive control group was able to emit light, as there was a cell-free system (TxTL Master Mix), GFP DNA, and an inducer (Inducer DNA) present in the sample. The first negative control group included the TxTL Master Mix (20 µL), GFP DNA (5 µL) (BioBuilder), and distilled water (1 µL). The first negative control group is unable to emit light as there was no inducer added. The second negative control group included the TxTL Master Mix (20 µL) (BioBuilder), distilled water (1 µL), and the cloning vector (5 µL). The second negative control group is unable to emit light as there was no inducer added. The experimental group included the TxTL Master Mix (20 µL), the Inducer DNA (5 µL) (BioBuilder), and the cloning vector (1 µL).

In the second trial, utilizing the BioBuilder lab *Ready, Set, Glow* kit and procedure, the cloning vector was tested with three groups: a negative control group, a positive control group, and an experimental group. The negative control group included one sample of the *TxTL Master Mix* (20 µL) (BioBuilder). The negative control group is unable to emit light as there is an absence of GFP DNA and inducer. The positive control

group included two samples of the *TxTL Master Mix* (20 μL), GFP DNA (5 μL), and *Inducer DNA* (5 μL) (BioBuilder). The experimental group included two samples of *TxTL Master Mix* (20 μL), *Inducer DNA* (BioBuilder), and the cloning vector.

Results

Tubes 1 and 2 are experimental tubes, tube 3 is a negative control, and tube 4 is a positive control. Tubes 1 and 2 held a lower particulate density than the positive control tube, but a higher particulate density than the negative control tube. This increase in density in our experimental tubes suggests that transcription and translation were present, as the chitinase protein is much larger than the particles in the Master Mix (amino acids and nucleotides).

Table 2.

	Particle Density
1 (experimental tube)	56927
2 (experimental tube)	54631
3 (negative control)	45065
4 (positive control)	76806

Discussions

Our results were not as we predicted, as CHIT1 was not expressed. As we expected, the two negative control groups did not emit light, and the positive control group did. Our experimental group, however, did not fluoresce as we had hoped. The reasoning for this is unclear. However, when reviewing our experiment, it seems that other materials produced cloudiness preventing the experimental group from fluorescing.

In both trials, the experimental groups displayed cloudiness that had particle densities lying in between the positive control groups (76806) and negative control groups (45065). This could suggest that, although the GFP gene was not expressed to the predicted extent, chitinase still could have been produced. This could have been because the experimental plasmid was a larger molecule than the GFP DNA in the positive control groups, and the kit used for the trials was not designed for a larger molecule. This, in turn, could have caused the GFP DNA in the experimental plasmid to have been unable to fold correctly. In turn, the chitinase gene in the experimental plasmid could have been expressed, which could explain the cloudiness.

Next steps

Currently, this work is only an engineering design for chitinase to be produced in a cell-free system. We have not yet tested whether this device can be transformed into a probiotic bacterial strain and successfully delivered to the intestinal lumen to alleviate the symptoms of IBD. Given additional time and resources, our research would continue by conducting further tests with this plasmid. Initially, this plasmid would be tested in *E. coli*, as it is easy to modify and test with. This would be followed by tests in *lactobacillus*, a bacterium already found in gut probiotics. Finally, there needs to be a way to encapsulate it to ensure its survival through the digestive system until it reaches the gut. This project lays the groundwork for further research in evaluating the use of chitinase to digest indigestible fibers, which will hopefully lead to better treatments for IBD.

Author contributions

J. A. and D. E. wrote the Background information. J. B. and C. S. wrote about the Materials and ethods used. R. B. and R. S. wrote the Results. E. O. and K. W. wrote the Discussion section.

Acknowledgements

We would like to acknowledge and thank our mentors, Eric Jorgenson and Dr. Jennifer Watson, as well as our supervision mentor/sponsor, Mr. Kirkpatrick. We appreciate their guidance, support, and help. We also want to thank Arbor Scientific, who provided us with the cell-free kits and allowed us to modify their T7-P14 GFP

plasmid, and K2 Scientific, who donated the -25-degree freezer, for providing us with the opportunity to pursue our interests in biology and making this project possible.

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