Can microbiota modulation prevent the development of fatty liver disease?

The BestTreat consortium is looking for answers.

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The term microbiota refers to the wide range of microorganisms (bacteria, parasites, fungi, and viruses) that populate a host. It has been acknowledged for decades that all living organisms inhabit specific collections of microbes on their different body parts. Although invisible to the naked eye, the human body carries ten times more microorganisms than human cells. Surprisingly, the microorganisms are estimated to have 100-times more genes than the human genome. Most of the human-associated microbiota is located in the gut, where the colon is the highest populated residency, with approximately 100 trillion microorganisms weighing as much as 1.5kg. The collection of microorganisms living on each body part is specific for that organ. For instance, the microbes living on our skin differ from those living in our mouths, which again differ from those living in our colon.

Gut microbes are responsible for many functions such as digesting, training our immune system and providing defence against pathogens. Microbiota dysregulation has been associated with severe and chronic diseases such as inflammatory bowel disease and Clostridium difficile infection. However, the microbial bacteria themselves are not the only players involved in health and disease. Molecules secreted or shredded from microbes may also influence the hosting organism by promoting health by, for example, providing essential nutrients and energy to our cells or introducing tissue damage via secreted toxins.

Could modifying some bacteria in our guts make the difference between health and disease?

Recent research has suggested that our microbiota is involved in developing various metabolic conditions and diseases such as obesity, diabetes and metabolic syndrome. Understanding the relationship between microbiota and disease development is necessary to advance therapeutic solutions. For this reason, a group of researchers joined the BestTreat consortium, a Marie-Skłodowska Curie Innovative Training Network, whose main aim is to identify microbial therapies for the treatment of non-alcoholic fatty liver disease (NAFLD).

NAFLD covers a range of disease stages, including liver fat accumulation. The mildest condition is named hepatic steatosis, which refers to the liver's simple and often non-recognised fat accumulation. In some subjects, hepatic steatosis develops into a more severe condition referred to as nonalcoholic steatohepatitis (NASH), which includes liver inflammation and fibrosis formation. Several risk factors like obesity, type 2 diabetes and metabolic syndrome have been identified, and NAFLD is one of the main reasons for liver transplantation worldwide.

How is this disease related to our gut microbiota and metabolites? And can we prevent or treat this disease by modulating the microbial composition in our gut?

To answer these questions, it is necessary to understand the interaction between gut microbiota and the liver. These two organs are closely connected since blood carrying both degraded food and microbial-derived compounds flows directly from the gut to the liver via the hepatic portal vein. The microbiota composition will influence their gut surroundings, promoting a healthy environment with a tight barrier that does not allow for the penetration of harmful components into the bloodstream. Alternatively, if pathogenic microbial overgrowth occurs, the barrier function may be damaged, leading to permeability of the intestinal tissue, which results in translocation of harmful components like lipopolysaccharide derived from Gram-negative bacteria. These harmful components will activate host immune cells in the bloodstream and organs such as the liver. Our continuous exposure to foreign bacteria, viruses, fungi and food particles, therefore, requires efficient defences to prevent penetration of external compounds into the blood and activation of the immune system.

The first line of defence is the gut epithelium: cells that form a physical barrier by separating the gut lumen from the bloodstream while at the same time allowing for absorption and transportation of compounds to the liver and other organs. Scientists agree on the important role the gut barrier plays in NAFLD disease. For instance, upon barrier disruption, unregulated compounds, such

as bacteria, metabolites and microbial debris rush through, reaching the bloodstream and eventually the liver. This triggers an inflammatory response in the liver that, when continued, contributes to the severity and duration of the disease.

Is it possible to protect the intestinal barrier before it gets damaged?

How can we strengthen the resilience and repair after the barrier has been disrupted during the disease?

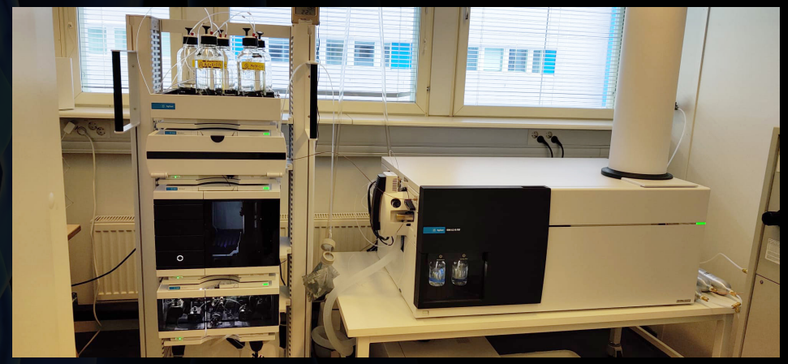
Is it possible to use bacteria in our intestine as microbial therapy for this task?

While researchers in the BestTreat consortium have identified several bacteria and bacterial metabolites that pose a risk for developing or progressing NAFLD, identifying beneficial bacteria and their associated metabolites has proven to be more challenging.

Our collaboration started at the Department of Molecular Genetics at the University of Groningen, where the Bacteroidetes phylum was carefully studied. It was speculated whether any of the strains of this phylum could have a beneficial impact on the gut barrier. Importantly, Bacteroidetes compose the majority of the Gram-negative bacterial population in the gut, and they are being proposed as next-generation probiotics (NGP). However, they are relatively understudied, although previous and current studies have indicated interesting antimicrobial activities, and there are genetic engineering tools designed for them. Therefore, a set of experiments were designed in which human cell lines, originating from both intestinal and hepatic tissue, were exposed to individual members of the phylum Bacteroidetes to identify bacterial candidates with beneficial effects against NAFLD development. We gathered a panel of 36 Bacteroidetes strains and explored their interaction with the epithelial human cell line HT-29. This cell line was used to identify the production of pro- and anti-inflammatory cytokines, a group of molecules involved in either activating or dampening inflammatory responses. The initial results showed that the immunomodulatory effect of the bacterial treatment was strain-dependent, with some strains promoting inflammation and others inhibiting inflammation. Out of the 36 initial candidates, we selected the top three bacterial performers that triggered anti-inflammatory responses and tested those for enhancement of the gut barrier function. This was done in collaboration with researchers from Chr. Hansen. We simulated colonic epithelial Caco-2 cells with the selected strains and observed which bacteria would strengthen or weaken the barrier integrity formed by these cells. This model simulates an in vivo situation wherein the gut epithelium is challenged by some strains and protected by others. Interestingly, two Bacteroides strains greatly enhanced the integrity of the Caco-2 cells monolayer, which suggests improvement in the gut barrier function. To sum up, we found two bacterial leads with immunomodulatory abilities, which could also help our gut by enhancing the intestinal barrier.

What about interaction with the liver?

In a disease state, metabolites and other bacterial-derived compounds pass through the hepatic portal vein and act directly on the liver. Thanks to the collaboration between the University of Eastern Finland and Chr. Hansen, we managed to set up a model to test the interaction between these molecules and liver cells. In this model, hepatocytes (the HepG2 cell line) were challenged with free fatty acids, leading to fat accumulation, which simulates the NAFLD condition. As bacteria are normally not in direct contact with the liver cells, we used the supernatant of overnight bacterial cultures of the selected strains containing the bacterial-derived metabolites. Preliminary results suggested that the supernatant of one of the Bacteroides strains reduced fat accumulation in the hepatocytes, which suggests alleviation of the NAFLD condition. Altogether, it seems that one out of the 36 initial Bacteroidetes strains seems to have promising results favouring a healthy gut barrier and protecting the hepatocytes from fatty acid accumulation. Although these initial experiments provided information about direct interaction between the bacterial and human cells, they did not address the multi-organ aspect of the disease. Our future collaboration will focus on this aspect and include more complex studies involving mouse models and clinical trials.



Metabolites are not only produced by microbes but may also originate from human host cells. Therefore, from each experimental study, we analysed the metabolome at Afekta Technologies, another partner of the BestTreat project. We used a non-targeted liquid chromatography-mass spectrometry-based approach to obtain the complete landscape of metabolites produced by both the bacteria and the different human cell lines. A preliminary analysis identified significantly increased microbial-derived metabolites, such as folic acid and tryptophan metabolites. Identifying the microbiota-derived metabolites and microbial-host metabolites through metabolomics provides new insights into the functional capacity of the gut microbiota interaction with human cells and helps understand the mechanisms behind such potentially beneficial microbial therapy.

Bringing together the different specialities from the various universities and companies not only strengthened the scientific knowledge gained in the project but also truly brought forward the spirit of collaboration in research and helped bridge the gap between academia and industry. Although it is still early days for the development of *Bacteroides* strains as microbial therapy for NAFLD in humans, we have, through in vitro screening, identified a strain with unique traits that will be further explored in animal trials for therapeutic efficacy against NAFLD development.

PROJECT NAME

BestTreat

PROJECT SUMMARY

The work described in this article is part of the Marie Skłodowska-Curie Innovative Training Network "Building a Gut Microbiome Engineering Toolbox for In-Situ Therapeutic Treatments for Nonalcoholic Fatty Liver Disease". We aim to develop new microbial therapies for the treatment of non-alcoholic fatty liver disease (NAFLD). Various strains from the Bacteroidetes phylum were investigated for beneficial traits protecting against NAFLD.

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