GABA Modulating Bacteria in the Human Gut Microbiome

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Abstract

Half of the bacterial species inhabiting the human intestinal tract will not grow in the laboratory. This is a significant unsolved problem, as our intestinal inhabitants have been linked to numerous gastrointestinal diseases, including Crohn’s disease, obesity, and type 2 diabetes. While important, these connections are perhaps not surprising since they are digestive disorders. An exciting new development is the unanticipated link of the microbiome to mental health, with the microbiome likely being involved in brain development, mood, and behavior, though specific mechanisms behind this communication are unknown. Previously, our group found that “uncultured” bacteria depend on neighboring “helper” bacteria for growth factors. In the present study, we used a similar co-culture approach to grow uncultured bacteria from human fecal samples, and successfully cultivated a number of organisms found on the NIH’s Most Wanted Genome List. One isolate, Flavonifractor sp., required the presence of Bacteroides fragilis or Dorea longicatena for growth. Using bio-assay driven purification of 8, fragilis supernatant, y-aminobutyric acid (GABA) was identified as the growth factor of Flavonifractor sp. GABA is the major inhibitory neurotransmitter of the mammalian central nervous system, and decreased levels are associated with depression and anxiety. Genomic analysis of Flavonifractor sp. suggests an unusual metabolic map focused on consuming a single nutrient, GABA. Using growth of Flavonifractor sp. as a bioassay, a number of abundant members of the gut microbiome were found to be producers of GABA. These GABA-modulating bacteria may be affecting mental health by consuming or producing this important neurotransmitter.

Background – The Gut-Brain-Axis

If one would compare the total number of bacterial cells on our skin or inside our bodies, cumulatively dubbed the microbiome, to the number of human cells we have, they would find that these microbes outnumber our own cells 10:1 (1). This begs us to ask the question, what exactly are we?

The majority of our resident bacteria are found in the gastrointestinal tract. Here there are an estimated 100 trillion bacterial cells made up of roughly 500 species, of which half can be cultured in the laboratory (2). Given their numbers, it is not surprising that these microorganisms have been found to play a role in nearly every human disorder, including obesity, cancer, and atherosclerosis (3). Recently, the microbiome has also been implicated to affect mental health, though the mechanism behind this gut-brain connection is unknown (4).

In this study, I reveal the discovery of a novel previously uncultured bacteria, Flavonifractor sp., and its growth factor, the neurotransmitter y-aminobutyric acid (GABA). I also show that abundant members of the gut microbiome produce large amounts of GABA. This data suggests that GABA-modulating bacteria could be impacting mental health, as decreased levels of GABA are associated with a variety of mental health disorders, including depression, anxiety, and autism. Consequently, therapeutic strategies could be designed around their introduction/removal.

Acknowledgements

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References


The GABA Consumer – Flavonifractor sp.

The GABA Producers – The Good Guys

Abstract

The GABA modulation by the gut microbiome may be a mechanism of communication. No Induction

Bifidobacterium longum

The GABA Producers

Clostridium scindens

Clostridium leptum

Flavonifractor

Bacteroides ovatus

Parabacteroides distasonis

Bacteroides vulgatus

Lactobacillus brevis

Bacteroides uniformis

Bacteroides caccae

Parabacteroides merdae

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Conclusions

- GABA is a growth factor for uncultured bacteria in the gut microbiome
- Flavonifractor sp. is an abundant member of the gut microbiome focused on fermenting GABA
- A number of the most abundant bacteria in the gastrointestinal tract produce large quantities of GABA
- GABA modulation by the gut microbiome may be a mechanism of communication along the gut-brain axis
- Introducing producers of GABA or eliminating Flavonifractor sp. could be therapeutic for mental health disorders

Figure 1. Isolation of the previously uncultured Flavonifractor sp. (A) Diluted fecal sample was plated on rich media and small, slow growing colonies were tested for dependence on larger, faster growing colonies. One isolate, Flavonifractor sp. was shown to be dependent on (B) Dorea longicatena or (C) Bacteroides fragilis for growth.

Figure 2. Identification of GABA as the growth factor of Flavonifractor sp. using bio-assay driven purification. Bacteroides fragilis supernatant was found to induce Flavonifractor sp. (A, B), and subsequent fractionation of supernatant by LC/MS lead to a single active fraction (C, D). NMR analysis identified GABA as the inducing factor (E, F).

Figure 3. Genomic analysis of Flavonifractor sp. The sequenced genome of Flavonifractor sp. was annotated using RAST (http://rast.nmpdr.org). Annotation revealed that Flavonifractor sp. is asaccharolytic and uses GABA for fermentation.

Figure 4. Ranking producers by GABA production. Several strains shown to induce (or not induce) Flavonifractor sp. were assayed for GABA production. After being grown for three days anaerobiically at 37 °C, cells were counted under a microscope and GABA was measured in the spent medium using LC/MS. GABA production was normalized for the number of cells, and all strains were tested in triplicate.