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GABA Modulating Bacteria – Can Our Bacteria Make Us Depressed?

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Abstract

The importance of the human gut microbiome (our gut bacteria) is hard to overstate -- our intestinal inhabitants have been linked to numerous gastrointestinal diseases, including Crohn's disease, obesity, and type II diabetes. While important, these connections are not surprising since they are digestive disorders. An exciting new development is the unanticipated link of the microbiome to mental health, with the microbiome being involved in brain development, mood, and behavior. 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. One isolate, KLE1738, required the presence of Bacteroides fragilis or Dorea longicatena for growth. Using bio-assay driven purification of B. fragilis supernatant, γ-aminobutyric acid (GABA) was identified as the growth factor of KLE1738. GABA is the major inhibitory neurotransmitter of the mammalian central nervous system, and decreased levels are associated with depression and anxiety. Genomic analysis of KLE1738 suggests an unusual metabolic map focused on consuming a single nutrient, GABA. Using growth of KLE1738 as a bioassay, a number of abundant members of the gut microbiome were found to be producers of GABA. We have also engineered one of the safest probiotics in the world, E. coli Nissle 1917, to produce GABA. By modulating levels of GABA modulating bacteria (by introducing our engineered E. coli Nissle 1917 strain or selectively targeting KLE1738), we may be able to design microbiome-based therapeutics to treat mental health disorders.

Background – The Gut-Brain-Connection

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).
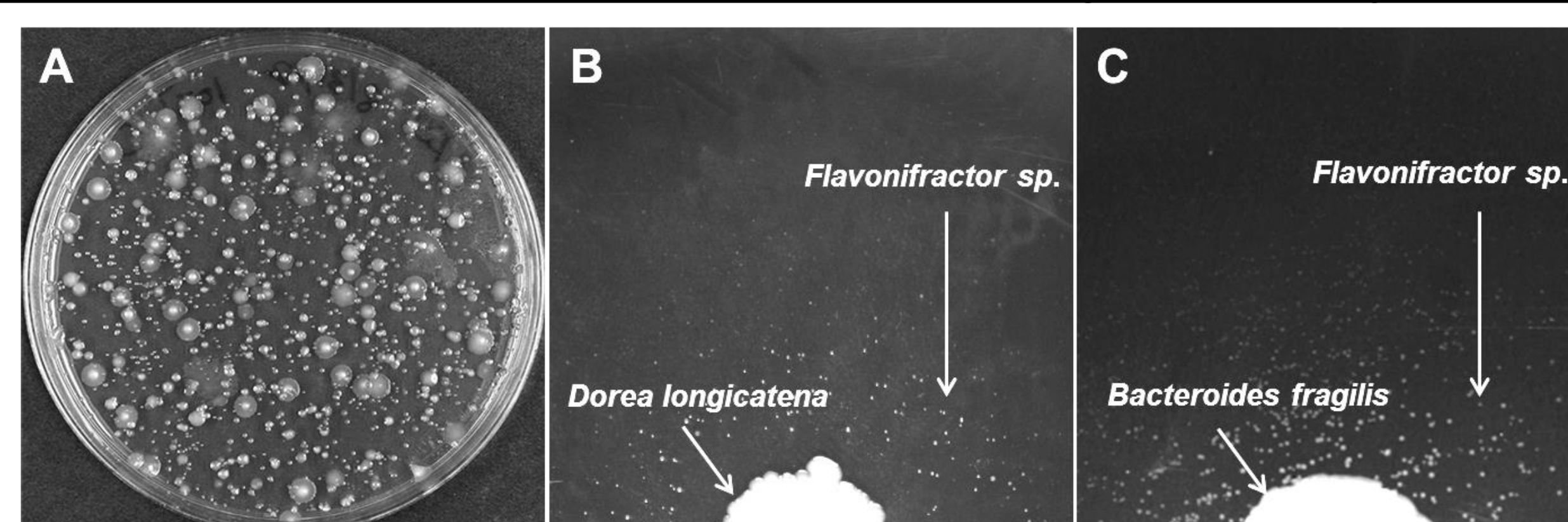
In this study, I reveal the discovery of a novel previously uncultured bacteria, *Flavonifractor* sp., and the identification of its growth factor, which is, surprisingly, the neurotransmitter γ-aminobutyric acid (GABA). I also show that abundant members of the gut microbiome produce large amounts of GABA. Cumulatively, this suggests that these 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, therapeutics could be designed around their introduction/removal.
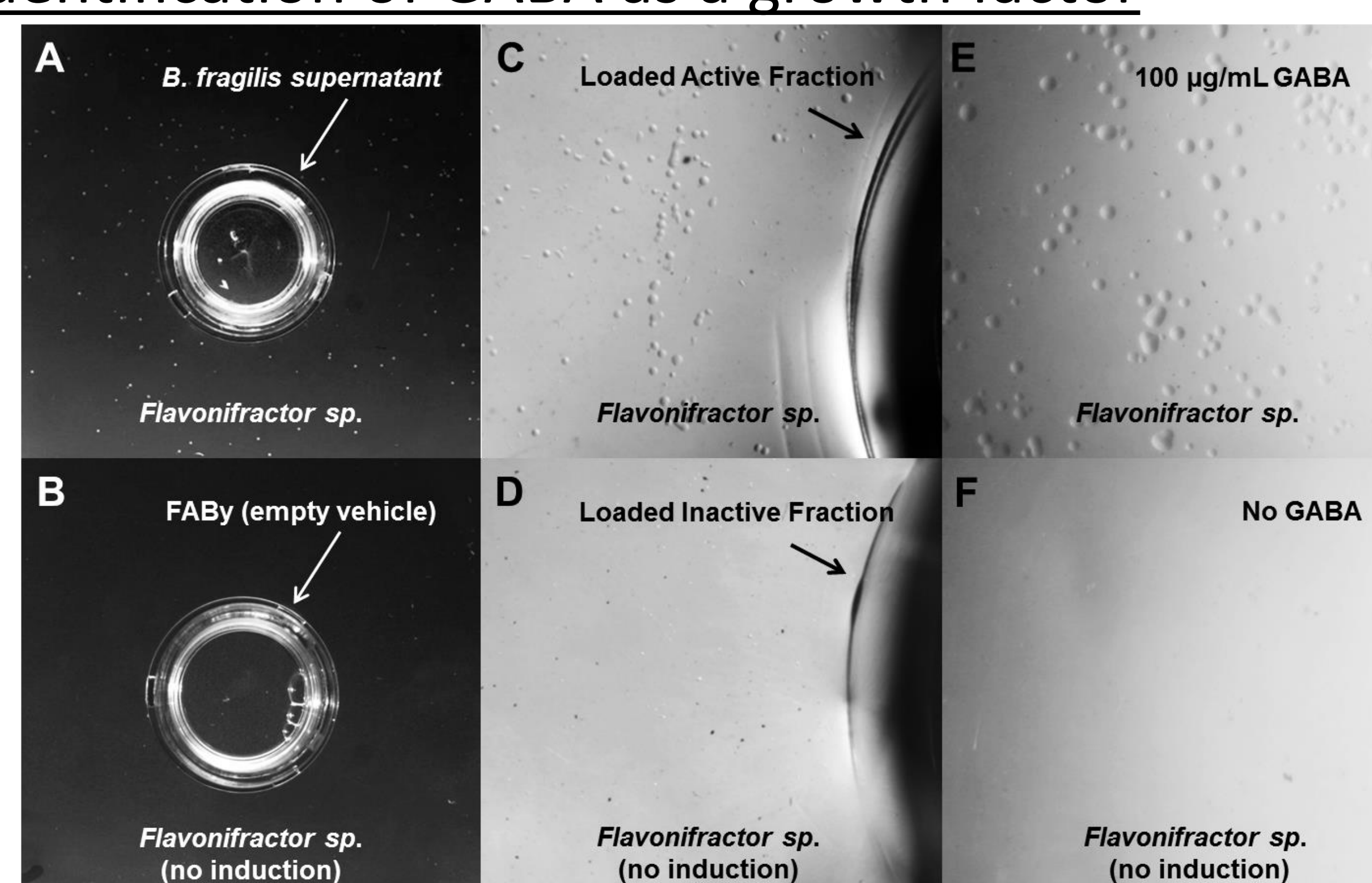
References

1. Belkaid, Y., Naik, S. 2013. Nat. Immunol.
2. Goodman, A.L., et al. 2011. PNAS.
3. Lozupone, C.A., et al. 2013. Nature.
4. Wang, Y., Kasper, L. 2014. Brain Behav Immun.

A GABA Consumer – A Depression Microbe?

Isolation of the uncultured *Flavonifractor* sp. KLE1738

A

B — *Flavonifractor* sp. / *Dorea longicatena*

C — *Flavonifractor* sp. / *Bacteroides fragilis*

Identification of GABA as a growth factor

A — *B. fragilis* supernatant / *Flavonifractor* sp.
B — FABy (empty vehicle) / *Flavonifractor* sp. (no induction)
C — Loaded Active Fraction / *Flavonifractor* sp.
D — Loaded Inactive Fraction / *Flavonifractor* sp. (no induction)
E — 100 μg/mL GABA / *Flavonifractor* sp.
F — No GABA / *Flavonifractor* sp. (no induction)

- Genome analysis found that KLE1738 was specialized on fermenting GABA
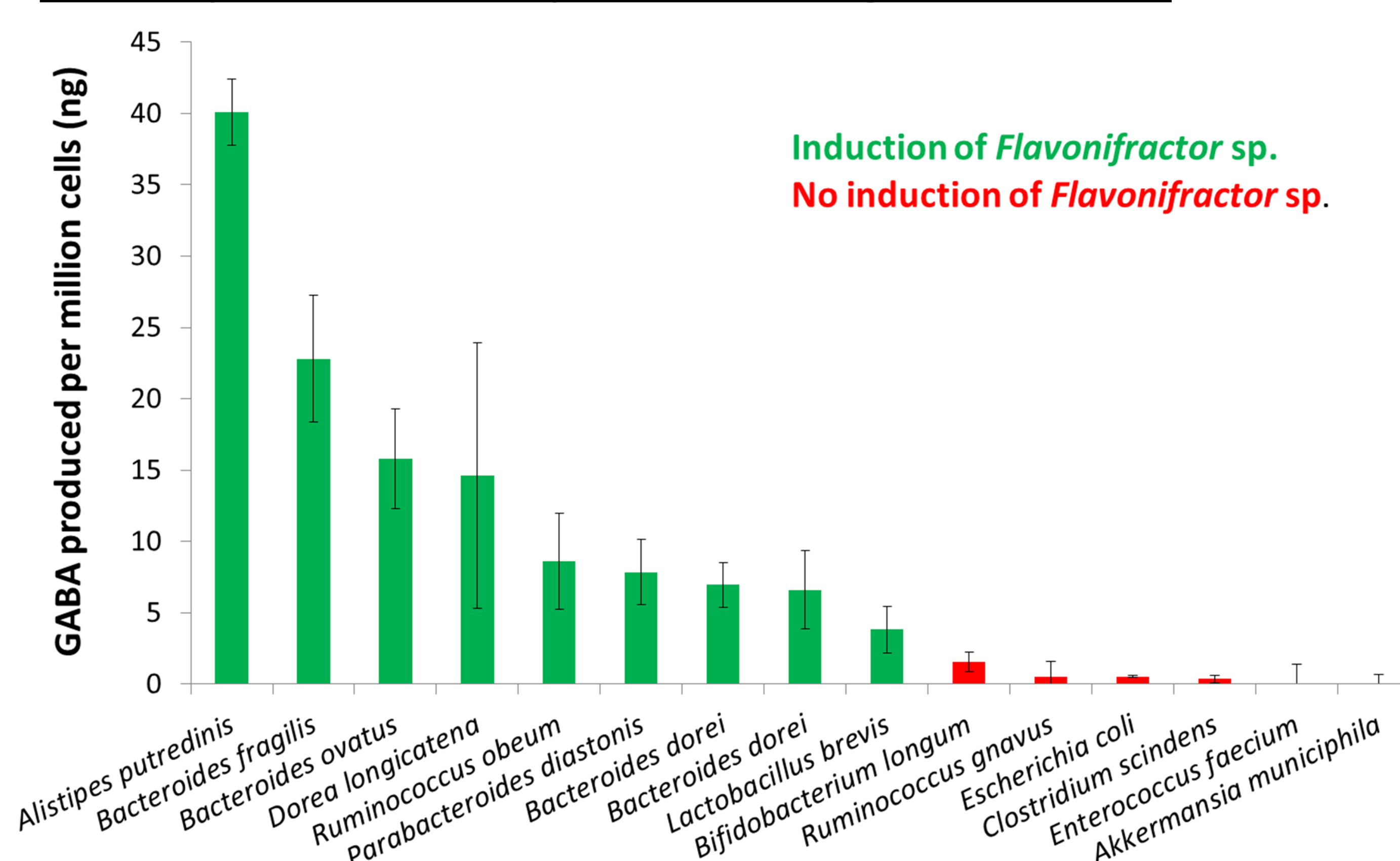
Forcing E. coli to produce GABA.

gadA   Empty Vector   *B. fragilis*

- By overexpressing glutamate decarboxylase (gadA) in *E. coli*, we can force it to produce GABA.
- We are currently engineering one of the safest probiotics in the world, E. coli Nissle 1917 to produce GABA.
- We will then test this strain in mouse behavioral models for anxiolytic/antidepressant effects

The GABA Producers – The Good Guys?

GABA production by abundant gut bacteria

Induction of *Flavonifractor* sp.
No induction of *Flavonifractor* sp.

Y-axis: GABA produced per million cells (ng)

X-axis: Alistipes putredinis, Bacteroides fragilis, Bacteroides ovatus, Dorea longicatena, Ruminococcus obeum, Parabacteroides diastonis, Bacteroides dorei, Bacteroides dorei, Lactobacillus brevis, Bifidobacterium longum, Ruminococcus gnavus, Escherichia coli, Clostridium scindens, Enterococcus faecium, Akkermansia municiphila

Conclusions

- GABA modulation by the gut microbiome may be a mechanism of communication along the gut-brain axis
- We identified a bacterium which is specialized to consume GABA, KLE1738
- As proof of principal, we can force E. coli to produce GABA, suggesting potential as a GABA-delivering organism.

What questions are we answering now?

- **Do these microbes influence mood/behavior?**
  - Introducing GABA-modulating bacteria into mice (McMaster)
- **What microbes (and microbial metabolites) are associated with depression/anxiety? Do GABA-modulating microbes play a role?**
  - Comparing the microbiomes of depressed/anxious individuals to healthy controls (NEU, MGH, Cornell Medical)
- **Can we find anxiolytic/antidepressant microbes, to use as drugs?**
  - Screening for microbes to protect against depression/anxiety
- **Can we use crowd funding to help fund this work?**
  - Launching a crowd sourcing campaign on Experiment.com to raise $100,000 (feel free to donate!)