The effects of per- and poly-fluoroalkyl substances (PFAS) on the beneficial gut bacterium *Lactobacillus acidophilus*

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ABSTRACT

Per- and poly- fluoroalkyl substances (PFAS) are a class of potentially harmful chemicals that can be introduced to the human body through ingestion and inhalation. PFAS are linked to disease in humans, but the exact mechanism of disease remains unknown. One of these pathways may be through damaging and inhibiting beneficial bacteria in the human gut. To determine if there may be a connection, we treated the beneficial bacteria *Lactobacillus acidophilus* with varying concentrations of 3 common PFAS- PFOA, PFOS, and GenX, and measured the impact on the growth of the bacteria using zones of inhibition and comparing bacterial growth curves. The data showed that the presence of PFAS will induce a change in the growth of *Lactobacillus* as seen in a growth curve, though no visible zones of inhibition resulted on agar plates for all three PFAS. Differences in the growth patterns of *Lactobacillus* indicate that PFAS presence in high levels in the body may inhibit beneficial bacterial growth in the gut and thus relate to human disease.

INTRODUCTION

PFAS contamination has recently become a large topic of discussion (and action) across the nation, specifically in the state of Michigan. PFAS are long, fluorinated carbon chain chemicals that were commonly used as food packaging, nonstick coating, firefighting foam, and as an important component of Teflon™. While PFOA and PFOS have been discontinued in the US, new, alternative options have taken their place, one of these being “GenX." These chemicals have found their way into ground water sources, and from there into the human body (Buck et al., 2011).

Because of this, and due to their connection with diseases, the CDC has named PFAS “chemicals of concern” (Anderson-Mahoney et al., 2008) (Center for Disease Control, 2018).
The exact mechanism of the diseases caused by PFAS is still unknown, though one option is through the disruption of beneficial bacteria in the gut. *Lactobacillus acidophilus* is a species of bacteria found in the human GI tract that has been shown to have major beneficial roles in human health. Damage to or imbalances of the microbiome have been linked to cancer, liver disease, diabetes, and more (Jandhyala et al., 2015) (Sekirov et al., 2010).

It is possible that long term exposure to PFAS in the GI tract through drinking water can interfere with or even kill bacteria in the gut, including the beneficial *Lactobacilli*. After a long period of exposure, alterations to the gut flora may occur, causing imbalances that previous research has associated with disease.

The purpose of this experiment is to investigate the relationship between PFAS and human health by determining the effect of PFAS on the beneficial bacteria of the gut.

OBJECTIVE AND HYPOTHESIS

Objective: To investigate the relationship between PFAS and human health by determining the effect of PFAS on *Lactobacillus acidophilus*

Hypothesis: It was hypothesized that all forms of PFAS (PFOA, PFOS, and GenX) would have a negative effect on *L. acidophilus*

METHODS

- *Lactobacillus acidophilus* culture
- *Escherichia coli* culture as control
  - known susceptibility to PFAS
- High and low concentrations of PFOA, PFOS, and GenX
  - representative of water samples
  - ranging from 3.44 x 10-6 M (1.72 mg/L, 1,720 ppt) to 2.135x10-3 M (920 mg/L, 920,000 ppt)
- Spectrophotometric assay
  - visualise how bacterial growth is affected over time
- Zones of inhibition
  - can determine if PFAS completely disrupts growth

RESULTS

- PFOA has a definitive negative effect on *L. acidophilus* (Table 1, Figure 2). This effect was not seen as strongly in *E. coli* (Table 1)
- Other PFAS not as much (Figure 3)
- GenX did not appear to have any worse (or better) outcomes than PFOS (Figure 3)

Table 1. Zone of inhibition diameter in *L. acidophilus* when treated with various PFAS

Trial number	High PFOA	High PFOS	High Gen X
1	17 mm	0	0
2	14 mm	0	0
3	16 mm	0	0
Average	15.66	0	0

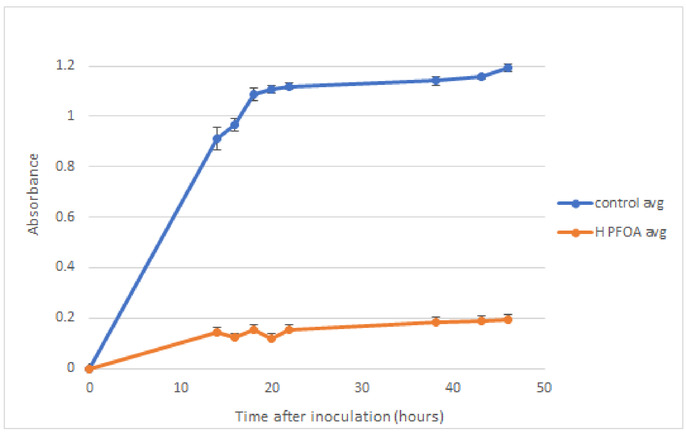

Figure 1. Absorbance of *E. coli* at 660 nm over the course of 34 hours when treated with a high PFOA concentration

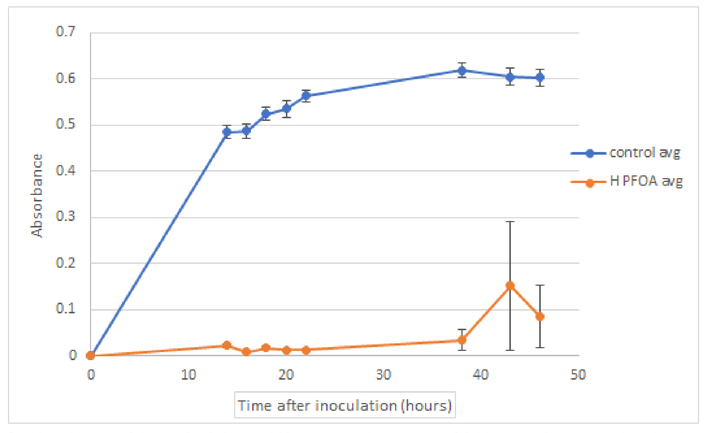

Figure 2. Absorbance of *L. acidophilus* at 660 nm over the course of 34 hours when treated with a high PFOA concentration

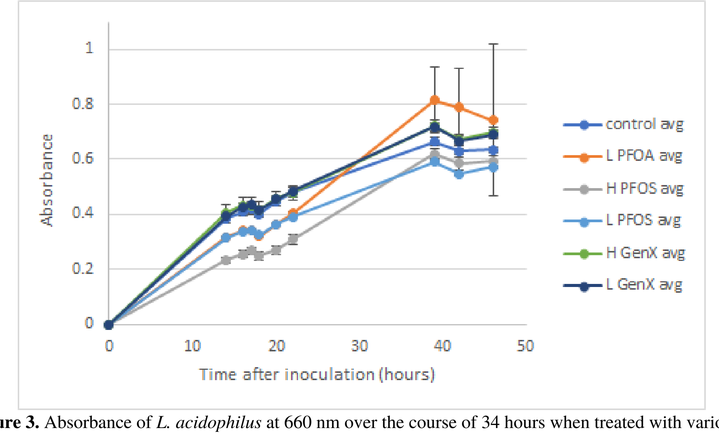

Figure 3. Absorbance of *L. acidophilus* at 660 nm over the course of 34 hours when treated with various PFAS

DISCUSSION

This investigation indicated that PFAS have an effect on the growth of *L. acidophilus,* particularly when treated with high concentrations of PFOA.

This increased sensitivity to PFOA, implying an increased toxicity of PFOA, is paralleled by data found by the Michigan PFAS Action Response Team, which recommended that the maximum contaminant level (MCL) of PFOA be the lowest of multiple PFAS, including PFOA, PFOS, and GenX (PFAS MCLS and Drinking Water, 2020).

The impact of PFAS on bacteria has previously been demonstrated in a 2016 study by Liu et al., stating that PFAS caused membrane disruption, oxidative stress, and DNA damage resulting in cell death or inactivation in bacteria (Liu et al., 2016). The cell death or inactivation observed in the 2016 study is mirrored in this study in the decreased growth curves of both *L. acidophilus* and *E. coli*, particularly when treated with PFOA.

The growth curve and zone of inhibition data for PFOS and GenX suggest that these PFAS do not have as strong of an effect on this particular species. Further testing involving a wider range of PFAS treatment concentrations could determine if PFOS or GenX do have some effect on *L. acidophilus*.

Combined with the knowledge that the growth and establishment of *L. acidophilus* plays a key role in human health, this study has supplied a potential linkage between PFAS and disease through the determination that PFAS may lead to the detriment of beneficial gut bacteria, and ultimately to deleterious effects on human health.

Future research should aim to isolate and further strengthen this connection between PFAS and gut health through testing the effects of PFAS on other beneficial bacteria in the gut that act cooperatively with *L. acidophilus*, in order to provide a full picture of the effects of PFAS on the gut microbiota and the human body in turn.

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