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# **The Effect of Artificial Sweeteners on *Lactobacillus acidophilus* and Its Potential Impact on the Female Endocrine System**

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# Research Questions

## Research Topic:

- Artificial sweeteners, the gut microbiome, and female endocrine health

## Rationale:

- To explore a potential biological mechanism linking the rising consumption of artificial sweeteners to disruptions in the gut microbiome and subsequent effects on female endocrine and reproductive health.

## Research Question:

- How does the consumption of artificial sweeteners affect the growth of *Lactobacillus acidophilus*, and what are the potential implications for female endocrine and reproductive health?

## Hypothesis:

Increasing concentrations of artificial sweeteners (Aspartame and Sucralose) will inhibit/slow the growth of *Lactobacillus acidophilus*. Reduced growth in vitro may suggest a mechanism through which NNS disrupts gut microbiome balance, potentially contributing to female endocrine and reproductive dysfunction.

# Key Concepts



Artificial Sweeteners (NNS):

- Non-caloric sugar substitutes (e.g., aspartame, sucralose)

Gut Microbiome:

- Community of microorganisms that regulate digestion, metabolism, and hormones

*Lactobacillus acidophilus*:

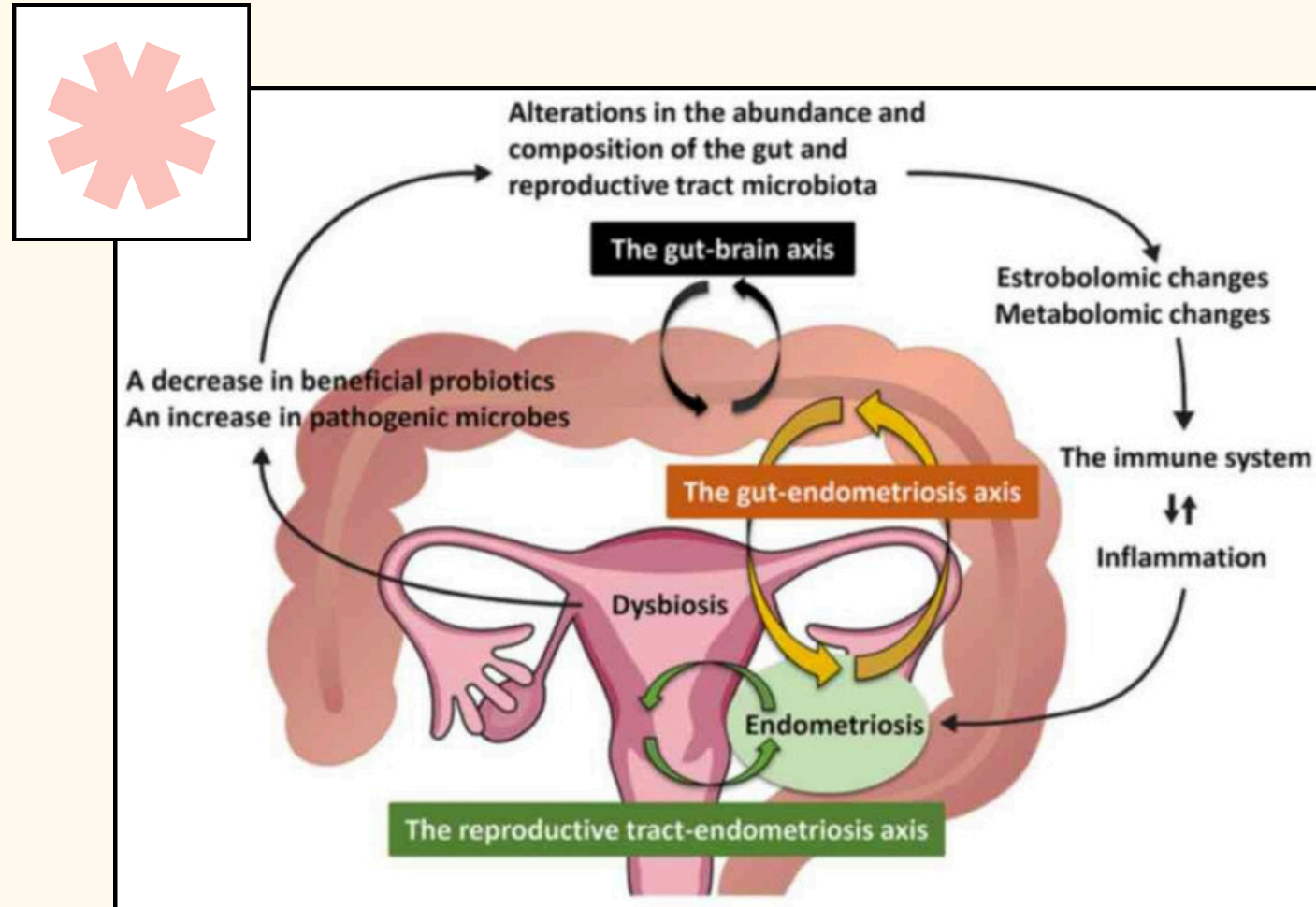
- Beneficial bacteria that maintain pH, inhibit pathogens, and support reproductive health

Gut–Vaginal Axis:

- Connection between gut microbiota and the female reproductive system

Dysbiosis:

- Imbalance in gut bacteria that can disrupt physiological functions

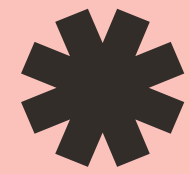


## The gut-vagina axis:

Proposed migration pathway for probiotic bacteria<sup>(9)</sup>



# Methodology



- In vitro experiment
- IV: sweetener types (Aspartame or Sucralose) & concentration (0, 20, 40, 100 mg/mL)
- DV: bacterial growth (**fractal dimension, Db**)
- Controlled variable: no sweetener exposure
- Controlled environmental variables → cause & effect clarity

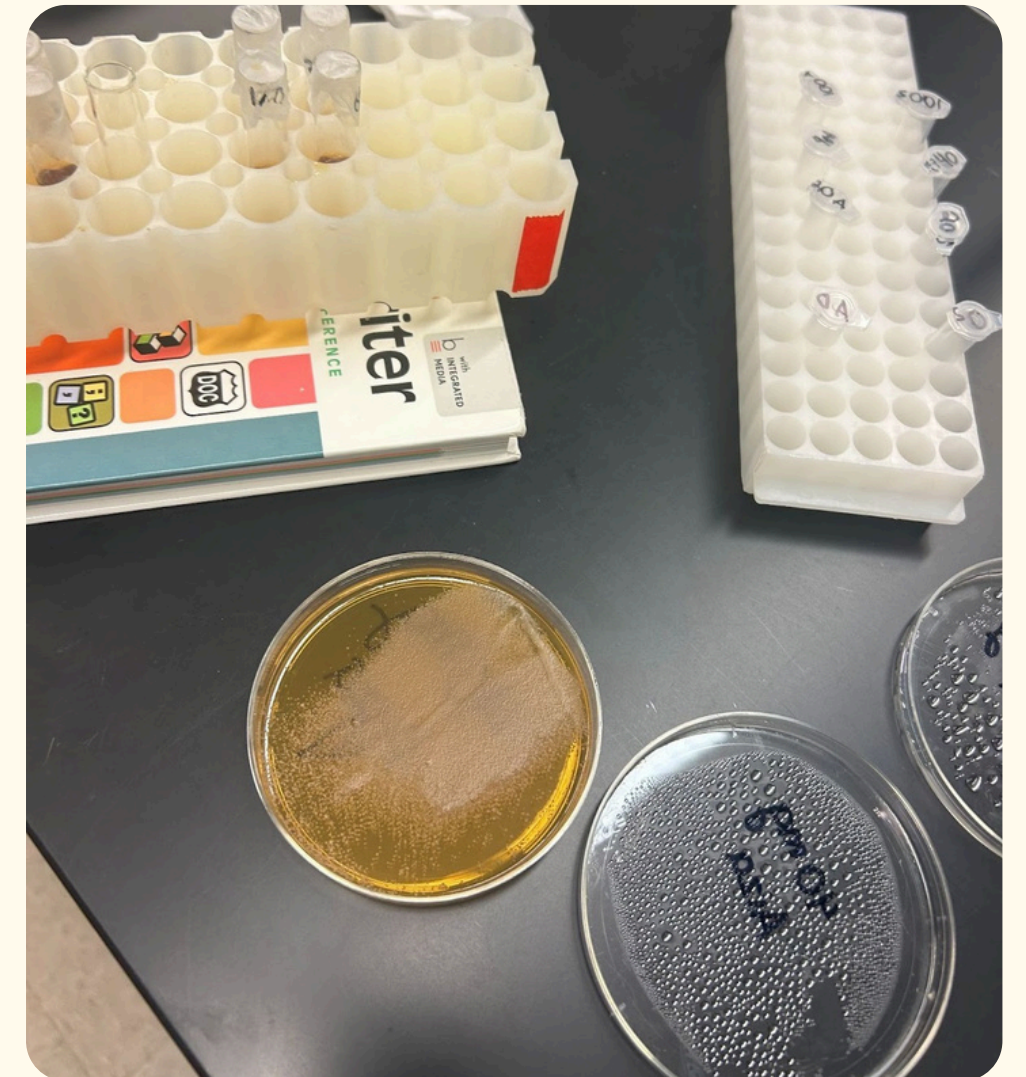


# Experimental Setup



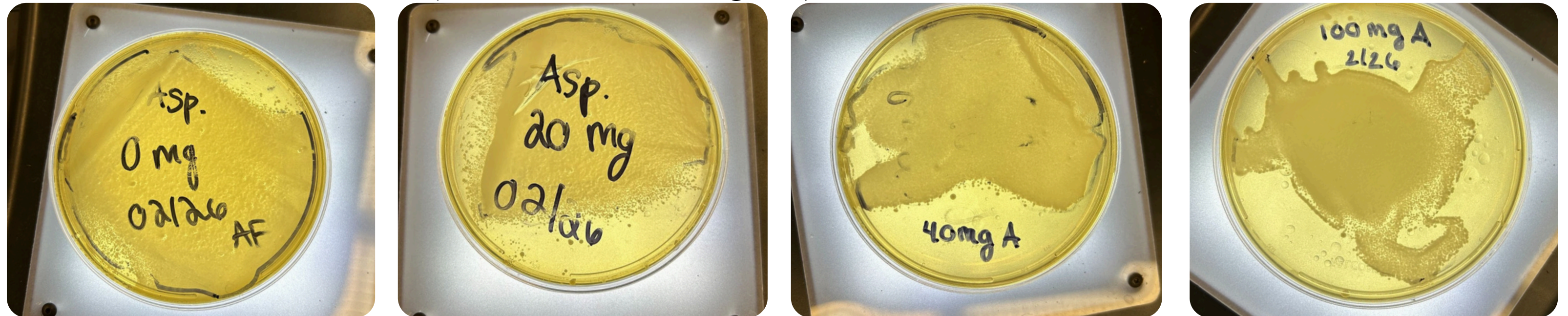
## Preparation + Procedure

- Prepare sweetener solutions
- Mix with *Lactobacillus* cultures
- Plate cultures on MRS agar plate
- Incubate 24 hours at 37°C
- Repeated trials
- Measure growth: **fractal dimension (Db)**



# Results: Colony Growth Images

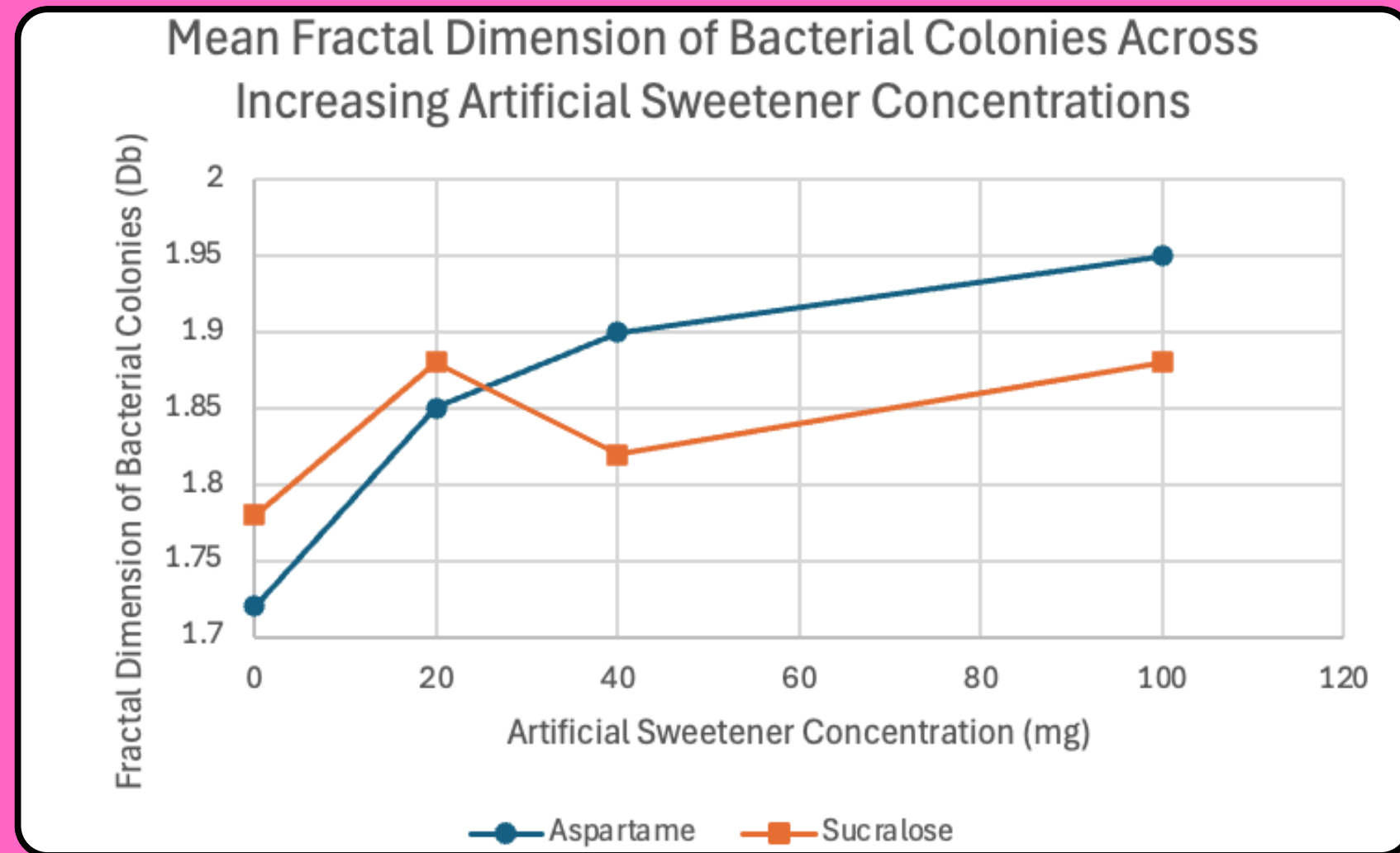
**Figure 1:** Representative growth of *Lactobacillus acidophilus* colonies on MRS agar at increasing aspartame concentrations (0, 20, 40, and 100 mg/mL) after 24 hours of incubation at 37 °C.



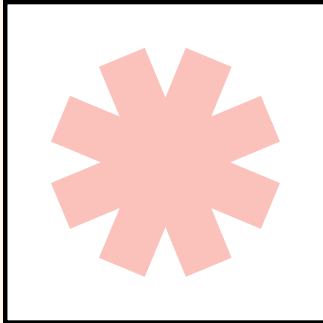
- Increasing aspartame and sucralose concentrations produced visible changes in *Lactobacillus acidophilus* colony growth patterns. Higher concentrations exhibited more irregular, dispersed colony structures than the control, consistent with the observed increase in fractal dimension (Db).

# Results: Fractal Dimension

Figure 2: Mean Db of Aspartame & Sucralose



- Increased concentration → disrupted growth patterns
- Higher Db = greater colony irregularity
- Similar trends for both sweeteners
- Suggests microbial stress or dysbiosis

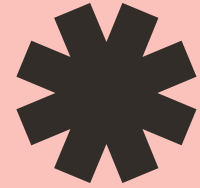


## Two-factor ANOVA analysis

Source	p-value
Sweetener Type	0.0635
Concentration	0.0079
Interaction	0.279

# Statistical Analysis

- Significance was determined using the alpha value of  $p < 0.05$
- Concentration:  $p = 0.0079$  (highly significant)
- Sweetener Type (Aspartame or Sucralose):  $p = 0.0635$  (insignificant)
- Interaction:  $p = 0.279$  (insignificant)



- Increasing sweetener concentration alters *Lactobacillus* growth
- Supports previous studies: artificial sweeteners → gut dysbiosis
- Potential downstream effect on female endocrine & reproductive health
  - Gut-vaginal axis
  - Fertility
  - Hormonal regulation

# Summary of Conclusions

Hypothesis supported

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# Applications & Future Research

## Applications

- Inform dietary guidelines for women of reproductive age regarding artificial sweetener intake
- Provide evidence for healthcare professionals to counsel patients on gut and reproductive health
- Highlight potential link between diet and fertility trends at a population level
- Serve as a foundation for further studies on microbiome-mediated endocrine effects

## Future Research

- Test additional artificial sweeteners (e.g., saccharin, stevia, Ace-K)
- Conduct in vivo studies
- Measure hormone levels and reproductive markers in conjunction with microbiome analysis
- Combined sweetener effects
- Use genetic and RNA analysis

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# Works Cited

- 1.) Alley, A. W., Paweł Łaniewski, Bruno, G. T., Moffitt, D. V., Gayatri Arani, Farland, L. V., & Herbst-Kralovetz, M. M. (2025). Vaginal Microbiome Dominated by Lactobacillus Positively Impacts Clinical Pregnancy in Patients With Frozen Embryo Transfers. *American Journal of Reproductive Immunology*, 93(6). <https://doi.org/10.1111/aji.70108>
- 2.) Driscoll, A., & Hamilton, B. (2025). Effects of Age-specific Fertility Trends on Overall Fertility Trends: United States, 1990-2023. *National Vital Statistics Reports*, 74. <https://www.cdc.gov/nchs/data/nvsr/nvsr74/nvsr74-3.pdf>
- 3.) Prevalence | UAMS Department of Pediatrics. (n.d.). <https://Medicine.uams.edu/Pediatrics/>. <https://medicine.uams.edu/pediatrics/specialties/sections/adolescent-medicine/patient-care/eating-disorders/prevalence/>
- 4.) Souza, S. V. de, Monteiro, P. B., Moura, G. A. de, Santos, N. O., Fontanezi, C. T. B., Gomes, I. de A., & Teixeira, C. A. (2023). Vaginal microbiome and the presence of Lactobacillus spp. as interferences in female fertility: A review. *JBRA Assisted Reproduction*, 27(3), 496–506. <https://doi.org/10.5935/1518-0557.20230006>
- 5.) Sylvetsky, A. C., Jin, Y., Clark, E. J., Welsh, J. A., Rother, K. I., & Talegawkar, S. A. (2017). Consumption of Low-Calorie Sweeteners among Children and Adults in the United States. *Journal of the Academy of Nutrition and Dietetics*, 117(3), 441-448.e2. <https://doi.org/10.1016/j.jand.2016.11.004>