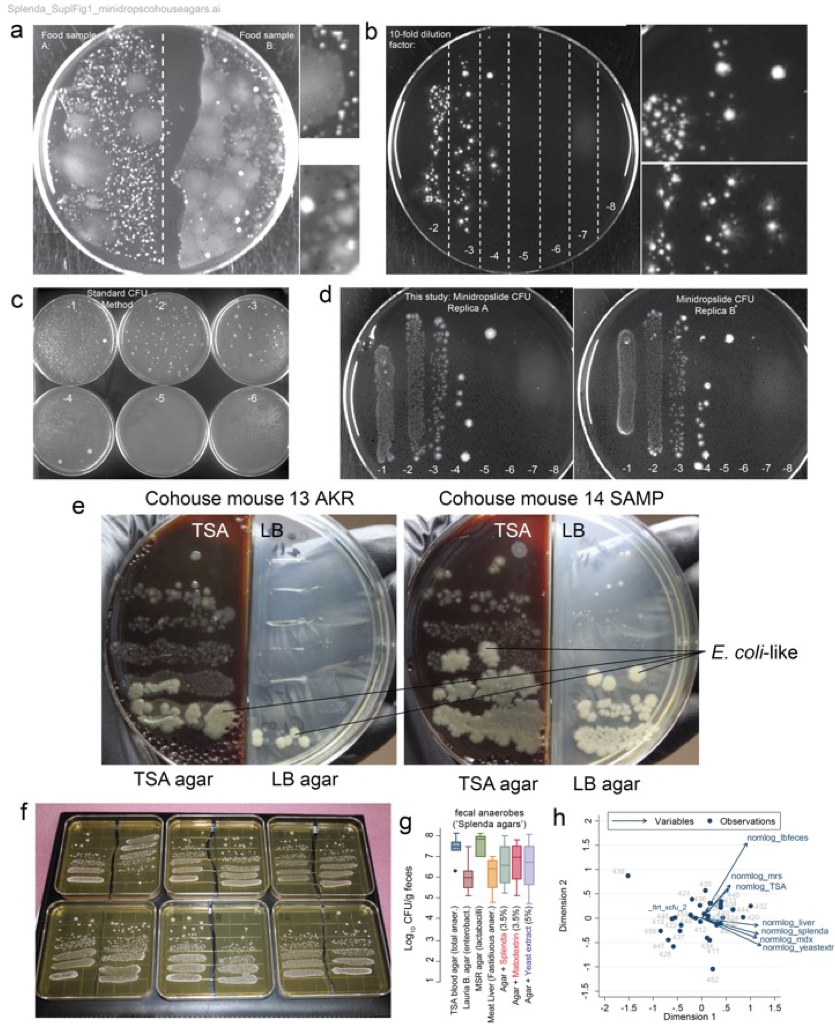
# The artificial sweetener Splen­­­­­da® promotes gut *Proteobacteria*, dysbiosis and myeloperoxidase reactivity in Crohn's disease-like ileitis

Alexander Rodriguez-Palacios, DVM, PhD,1 Andrew Harding, MD,1 Paola Menghini, PhD,1 Catherine Himmelman,1 Mauricio Retuerto, BSc,2 Kourtney P. Nickerson, PhD,3 Minh Lam, PhD,1 Colleen M. Croniger, PhD,4 Mairi H McLean, MBChB PhD,6,7 Scott K. Durum, PhD,6 Theresa T. Pizarro, PhD,5 Mahmoud A. Ghannoum, PhD,2 Sanja Ilic, PhD,8 Christine McDonald, PhD,3 and Fabio Cominelli, MD, PhD1,9

**Supplementary Material:**

* Supplementary Figure 1.
* Supplementary Table 1.



## Supplementary Figure 1: Development of a ‘Parallel Lanes Platting’ method for relative enumeration of bacteria in complex communities.

**a**) Two food samples (retail ground meat) on TSA illustrate diverse bacterial colonies at different concentrations in a relatively simple community (100 μL/half plate). **b**) ‘Parallel minidrop lanes platting’ of *Clostridium difficile* monocultures (10-20 μL/lane) illustrates that several solutions can be incubated on a single agar plate and quantified based on colony morphology. TSA and UV transilluminator. Multichannel pipettes allow the transfer of 8 dilutions on a single media plate. **c**) Standard plating methods use 100 μL/plate, making it agar intensive and difficult to track as the number of plates increase. **d**) The parallel minidrop lanes platting method is highly reproducible. **e**) Herein, we illustrate the use of the method on fecal samples from two cohoused mice in TSA and LB agars. Notice that SAMP has more *E. coli* bacteria relative to the total number of bacteria. **f**) The same approach in square plates was used for Splenda® experiments to increase ability to select single colonies for PCR. MRS agar (lactobacilli). **g**) ‘Parallel Lanes Platting’ of fecal samples using in house agars (‘maltodextrin and Splenda® agars’). **h**) Multivariable analysis revealed that several agars yielded similar bacterial counts to assess the effect of Splenda® on SAMP mice. Meat liver base agar enables the quantification of strict and fastidious anaerobes, while allows (e.g., some *Clostridium* spp.) sulfite reducers to produce hydrogen sulfide (H2S), which allows the blackening of colonies due to presence of ferric ammonium citrate.

|  |  |
| --- | --- |
| |  | | --- | | **Supplementary Table 1**. Summary of MPO activity in tissues of mice after supplementation with Splenda® | |
| |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | | SAMP1/YitFc | Treatment | N | Minimum | Q1 | Median | Q3 | Maximum | 95% Median CI | | Colon | H2O | 7 | 5.133 | 6.372 | 8.437 | 10.560 | 16.991 | (6.041, 12.275) | |  | Splenda® 3.5% | 7 | 3.127 | 3.481 | 7.316 | 10.855 | 23.245 | (3.386, 14.159) | | Ileum | H2O | 7 | 79.35 | 92.30 | 103.72 | 150.27 | 153.22 | (88.85, 151.05) | |  | Splenda® 3.5% | 7 | 70.68 | 124.54 | 172.39 | 223.83 | 294.28 | (110.18, 242.62) | |
| |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | | AKR/J mice |  |  |  |  |  |  |  |  | | Colon | H2O | 5 | 2.5369 | 2.5959 | 3.1268 | 3.6283 | 3.7758 | (2.5369, 3.7758) | |  | Splenda® 3.5% | 6 | 0.5310 | 2.6106 | 4.1003 | 6.6077 | 6.6077 | (1.5213, 6.6077) | | Ileum | H2O | 5 | 3.07 | 3.07 | 3.07 | 46.17 | 48.97 | (3.07, 48.97) | |  | Splenda® 3.5% | 6 | 5.192 | 7.670 | 12.611 | 48.230 | 53.540 | (6.372, 51.011) | |