1. **Supplemental Microbiome Analyses Methods**

Fecal samples were collected by participants with OMNIgene Gut collection tubes (OM-200, OMR 200) provided by DNA genotek (Ottawa, ON, Canada). Tubes were labelled with study ID number, sample ID number, randomization number and visit. After collection, the participants kept the samples at room temperature (20–26 °C) and submitted the collection tubes to the site lab within 3 days from the collection. The stool samples were kept in a safe, well-ventilated area at room temperature until they were stored in the lab freezer at –80 ± 10 °C, before being transferred to the analytical laboratory on dry ice.

Microbiome analyses were conducted by Microbiome Insights (Richmond, British Columbia), where fecal samples were stored in a -70 °C freezer without any additional buffer. Specimens were placed into a Mo Bio PowerMag Soil DNA Isolation Bead Plate. DNA was extracted following Mo Bio’s instructions on a KingFisher robot (Mo Bio Laboratories). Bacterial 16S rRNA was PCR-amplified with dual barcoded primers targeting the V4 region (515F 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′) (as described in Kozich *et al*., 2013). Amplicon sequencing of the 16S regions was done with Illumina MiSeq using the 300bp paired end kit (v3). Relative abundance methods were applied with MiSeq Reagent Kit v3.0 running in a 250 bp read length, paired end (aka 2×250 bp).

For quality control, the operational taxonomic unit were considered putative contaminants (and were removed) if their mean abundance in controls reached or exceeded 25% of their mean abundance in the specimens. No low quality read/samples were identified. Contaminate mitigation was addressed by co-sequencing DNA amplified from specific and from template-free controls. A positive control from ‘S00Z1-’ samples consisting of cloned SUP05 DNA was included.

Taxonomic classification was done using Silva (v 138.1) as the reference database, and sequences were clustered in 97% similarity operational taxonomic units (OTUs) with the mothur software package (V1.48.0) (see Schloss et al. for details). The resulting dataset had 21505 OTUs. An average of 47009 quality filtered reads were generated per sample. Sequencing for R1 and R2 was determined using FastQC 0.11.5. OTUs were aggregated into each taxanomic rank (phylum, class, order family, genus).

Alpha diversity was estimated with the Shannon index on raw OTU abundance. Linear mixed model was used to test significance of diversity differences. Bray-Cutis indices were computed to estimate beta diversity after excluding OTUs occurring with a count of less than 3 in at least 10% of samples. Variation in community structure was assessed with permutational multivariate analyses of variance with treatment group as the main fixed factor and using permutations for significance testing. All analyses were conducted in R environment.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D. 2013. Develop of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology. 79: 5112-5120.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology. 75: 7537-7541.

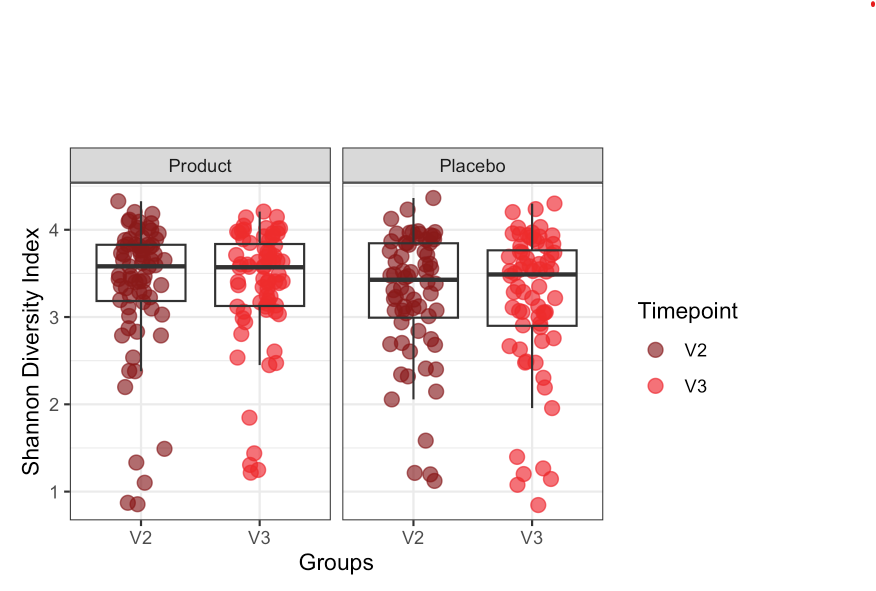
**a)** A green and yellow vertical lines

Description automatically generated

**b)** A screenshot of a graph

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SUPPLEMENTARY FIGURE S1. Phylum (a) and Genus (b) relative abundances were not different between groups at any visit.Product: B. coagulans Unique IS2.



SUPPLEMENTARY FIGURE S2. Shannon-alpha Diversity indices in Product (B. coagulans Unique IS2) and Placebo at each visit.There were no differences observed between the two study products at either visit with respect to Shannon diversity indices. Product: B. coagulans Unique IS2.