

Supplementary Information

Title

Effects of Yeast Mannan which Promotes Beneficial *Bacteroides* on the Intestinal Environment and Skin Condition: A Randomized, Double-Blind, Placebo-Controlled Study

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Table S1. qPCR amplification conditions for each bacterial species.

| Target species | Primer-F Primer-R (5' to 3') Probe (5' FAM - 3' TAMRA) | Strains for standard curves | PCR amplification condition | Ref. |
|----------------------------|---|--|--|------|
| All eubacteria | CGGTGAATACGTTCCCGG TACGGCTACCTTGTTACGACTT | <i>B. fragilis</i> ATCC 25285 | 95 °C for 10 s, [95 °C for 20 s, 56 °C for 20 s, and 72 °C for 30 s] × 40 cycles | 24 |
| <i>B. thetaiotaomicron</i> | GCAAACCTGGAGATGGCGA AAGGTTTGGTGAGCCGTTA TCGATGGGGATGCGTTCCATTAGG | <i>B. thetaiotaomicron</i> ATCC 29741 | 95 °C for 30 s, [95 °C for 15 s and 62.5 °C for 60 s] × 40 cycles | 23 |
| <i>B. ovatus</i> | TGCAAACCTRAAGATGGC CAAACCTAATGGAACGCATC CACGTATCCAACCTGCCGATAACTCC | <i>B. ovatus</i> ATCC 8483 | 95 °C for 30 s, [95 °C for 15 s and 58 °C for 60 s] × 40 cycles | 23 |
| <i>B. vulgatus</i> | CGGGCTTAAATTGCAGATGA CATGCAGCACCTTCACAGAT TGAAAGCCGTAAGCCGCAAGG | <i>B. vulgatus</i> ATCC 8482 | 95 °C for 30 s, [95 °C for 15 s and 63 °C for 60 s] × 40 cycles | 23 |
| <i>B. uniformis</i> | TCTTCCGCATGGTAGAACTATTA ACCGTGTCTCAGTTCCAATGTG CGTTCCATTAGGTTGTTGGCGGGG | <i>B. uniformis</i> ATCC 8492 | 95 °C for 30 s, [95 °C for 15 s and 60 °C for 60 s] × 40 cycles | 23 |
| <i>B. fragilis</i> | TCRGAAGAAAGCTTGCT CATCCTTTACCGGAATCCT ACACGTATCCAACCTGCCCTTACTCG | <i>B. fragilis</i> ATCC 25285 | 95 °C for 30 s, [95 °C for 15 s and 63 °C for 60 s] × 40 cycles | 23 |

Table S2. Comparison of relative abundance of *Bacteroides* species between the YM and placebo group.

| Species | Treatment | Changes from baseline | |
|------------------------------|-----------|-----------------------|--------------|
| | | 4 Weeks | 8 Weeks |
| <i>B. thetaiotaomicron</i> * | YM | 1.45 ± 0.76 | 0.51 ± 0.17 |
| | Placebo | -0.07 ± 0.15 | 0.13 ± 0.14 |
| <i>B. ovatus</i> * | YM | 1.81 ± 0.74 | 1.09 ± 0.38 |
| | Placebo | 0.28 ± 0.43 | 0.16 ± 0.28 |
| <i>B. vulgatus</i> | YM | 0.59 ± 0.33 | 0.28 ± 0.35 |
| | Placebo | 0.23 ± 0.23 | 0.23 ± 0.20 |
| <i>B. uniformis</i> | YM | 0.40 ± 0.26 | 0.31 ± 0.36 |
| | Placebo | -0.06 ± 0.32 | 0.12 ± 0.32 |
| <i>B. fragilis</i> | YM | 0.09 ± 0.05 | 0.02 ± 0.03 |
| | Placebo | -0.06 ± 0.14 | -0.11 ± 0.13 |

Data represent the mean ± standard error (SE). Asterisks indicate overall significance between groups across time points measured using repeated measures ANOVA for the changes from baseline without multiple comparison correction (* $p < 0.05$).

Table S3. Comparison of bowel habits and fecal conditions between the YM and placebo group.

| Item | Unit | Treatment | Baseline | | 4 Weeks | | 8 Weeks | |
|------------------------|------------|-----------|----------|-------|---------|-------|---------|-------|
| Defecation days * | days/week | YM | 5.3 | ± 0.2 | 5.5 | ± 0.2 | 5.6 | ± 0.2 |
| | | Placebo | 5.4 | ± 0.2 | 5.2 | ± 0.2 | 5.3 | ± 0.2 |
| Defecation times | times/week | YM | 5.9 | ± 0.2 | 6.1 | ± 0.2 | 6.5 | ± 0.3 |
| | | Placebo | 5.7 | ± 0.2 | 5.7 | ± 0.2 | 5.9 | ± 0.2 |
| BSS | | YM | 3.8 | ± 0.1 | 3.8 | ± 0.1 | 3.9 | ± 0.1 |
| | | Placebo | 3.8 | ± 0.1 | 3.9 | ± 0.1 | 3.8 | ± 0.1 |
| Fecal water contents** | % | YM | 72.4 | ± 1.0 | 74.3 | ± 1.0 | 73.4 | ± 0.9 |
| | | Placebo | 74.7 | ± 0.9 | 72.7 | ± 0.9 | 72.4 | ± 0.9 |

Data represent the mean ± SE. BSS: Bristol Stool Scale. Asterisks indicate overall significance between groups across time points measured using repeated measures ANOVA for the changes from baseline without multiple comparison correction (* $p < 0.05$, ** $p < 0.01$).

Table S4. Comparison of CAS scores between the YM and placebo group.

| Item | Treatment | Baseline | | 4 Weeks | | 8 Weeks | |
|---|-----------|----------|--------|---------|--------|---------|--------|
| Inability to pass stool** | YM | 0.43 | ± 0.08 | 0.20 | ± 0.07 | 0.27 | ± 0.06 |
| | Placebo | 0.33 | ± 0.07 | 0.39 | ± 0.07 | 0.41 | ± 0.07 |
| Less frequent bowel movements* | YM | 0.08 | ± 0.04 | 0.08 | ± 0.04 | 0.04 | ± 0.03 |
| | Placebo | 0.08 | ± 0.04 | 0.18 | ± 0.06 | 0.16 | ± 0.05 |
| Small volume of stool* | YM | 0.14 | ± 0.05 | 0.06 | ± 0.03 | 0.04 | ± 0.03 |
| | Placebo | 0.04 | ± 0.03 | 0.10 | ± 0.04 | 0.08 | ± 0.04 |
| Abdominal distention or bloating | YM | 0.31 | ± 0.07 | 0.33 | ± 0.07 | 0.31 | ± 0.07 |
| | Placebo | 0.45 | ± 0.08 | 0.25 | ± 0.06 | 0.35 | ± 0.07 |
| Change in amount of gas passed rectally | YM | 0.27 | ± 0.08 | 0.33 | ± 0.08 | 0.20 | ± 0.07 |
| | Placebo | 0.18 | ± 0.07 | 0.12 | ± 0.05 | 0.18 | ± 0.07 |
| Rectal fullness or pressure | YM | 0.39 | ± 0.08 | 0.27 | ± 0.06 | 0.24 | ± 0.06 |
| | Placebo | 0.31 | ± 0.07 | 0.39 | ± 0.07 | 0.31 | ± 0.07 |
| Rectal pain with bowel movements | YM | 0.10 | ± 0.04 | 0.12 | ± 0.05 | 0.16 | ± 0.05 |
| | Placebo | 0.06 | ± 0.03 | 0.06 | ± 0.03 | 0.12 | ± 0.05 |
| Oozing liquid stool | YM | 0.10 | ± 0.04 | 0.10 | ± 0.04 | 0.12 | ± 0.05 |
| | Placebo | 0.16 | ± 0.05 | 0.12 | ± 0.05 | 0.14 | ± 0.05 |
| Total CAS score | YM | 1.82 | ± 0.27 | 1.49 | ± 0.24 | 1.39 | ± 0.20 |
| | Placebo | 1.61 | ± 0.22 | 1.61 | ± 0.23 | 1.75 | ± 0.22 |

Data represent the mean ± SE. CAS: Constipation assessment scale. Asterisks indicate overall significance between groups across time points measured using repeated measures ANOVA for the changes from baseline without multiple comparison correction (* $p < 0.05$, ** $p < 0.01$).

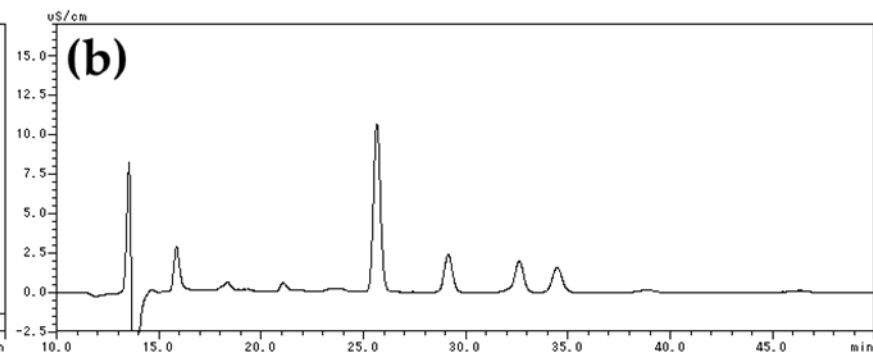
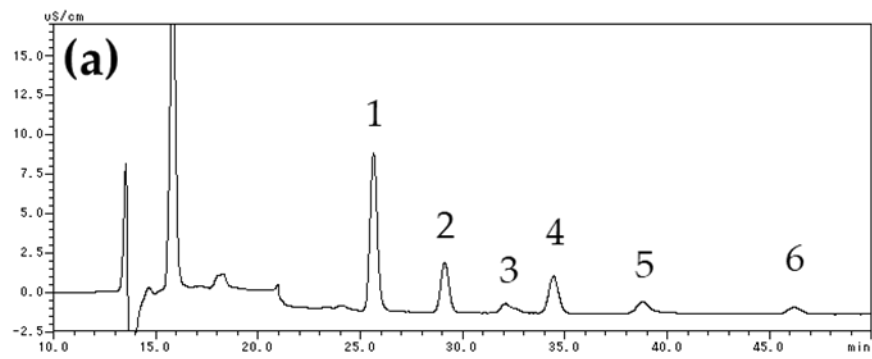
Table S5. Comparison of fecal putrefactive compound levels between the YM and placebo group.

| Fecal metabolite | Unit | Treatment | Changes from baseline | | | |
|-----------------------------|------------|-----------|-----------------------|--------|---------|--------|
| | | | 4 Weeks | | 8 Weeks | |
| Indole** | µg/g-feces | YM | -10.8 | ± 5.2 | -0.3 | ± 5.0 |
| | | Placebo | 8.5 | ± 3.3 | 10.0 | ± 3.2 |
| <i>p</i> -Cresol* | | YM | -21.0 | ± 12.6 | 9.2 | ± 16.8 |
| | | Placebo | 24.4 | ± 8.6 | 28.3 | ± 9.0 |
| Skatole* | | YM | -1.2 | ± 1.1 | -0.8 | ± 2.0 |
| | | Placebo | 2.2 | ± 1.3 | 2.8 | ± 1.2 |
| Phenol | | YM | -2.1 | ± 1.9 | -2.6 | ± 1.5 |
| | | Placebo | -1.9 | ± 1.0 | -2.1 | ± 1.7 |
| 4-Ethylphenol | | YM | -0.1 | ± 0.6 | -0.2 | ± 0.5 |
| | | Placebo | 0.3 | ± 0.5 | 0.2 | ± 0.5 |
| Sum of phenols and indoles* | YM | -35.2 | ± 17.3 | 5.4 | ± 22.4 | |
| | Placebo | 33.5 | ± 11.5 | 39.3 | ± 12.1 | |
| Ammonia* | YM | -59.4 | ± 49.5 | 60.3 | ± 50.4 | |
| | Placebo | 61.9 | ± 36.4 | 187 | ± 49.2 | |

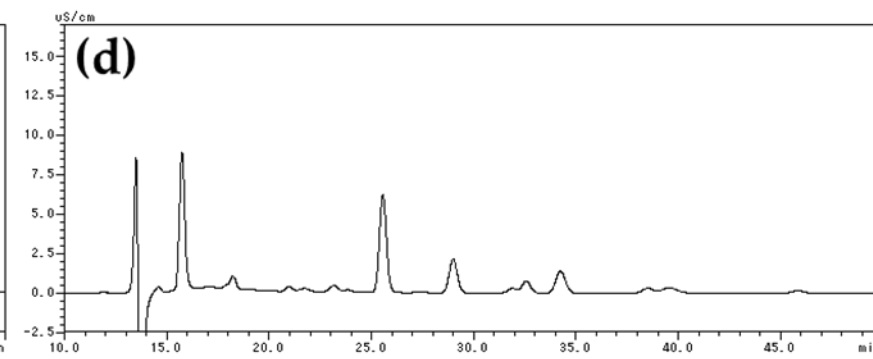
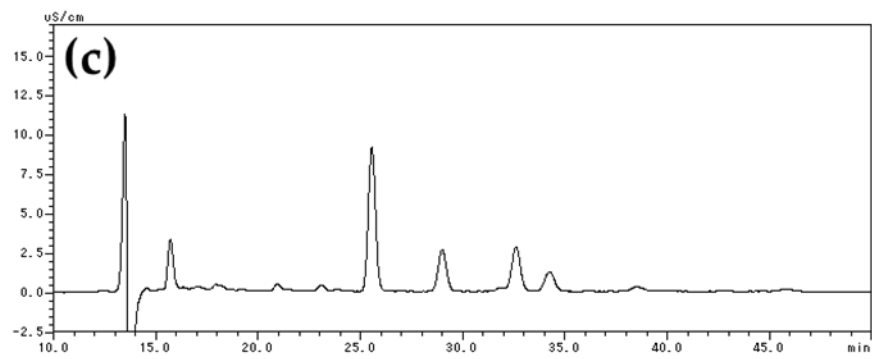
Data represent the mean ± SE. Asterisks indicate overall significance between groups across time points measured using repeated measures ANOVA for the changes from baseline without multiple comparison correction (* $p < 0.05$, ** $p < 0.01$).

YM group

Placebo group



0 Weeks



8 Weeks

Figure S1. (Continued)

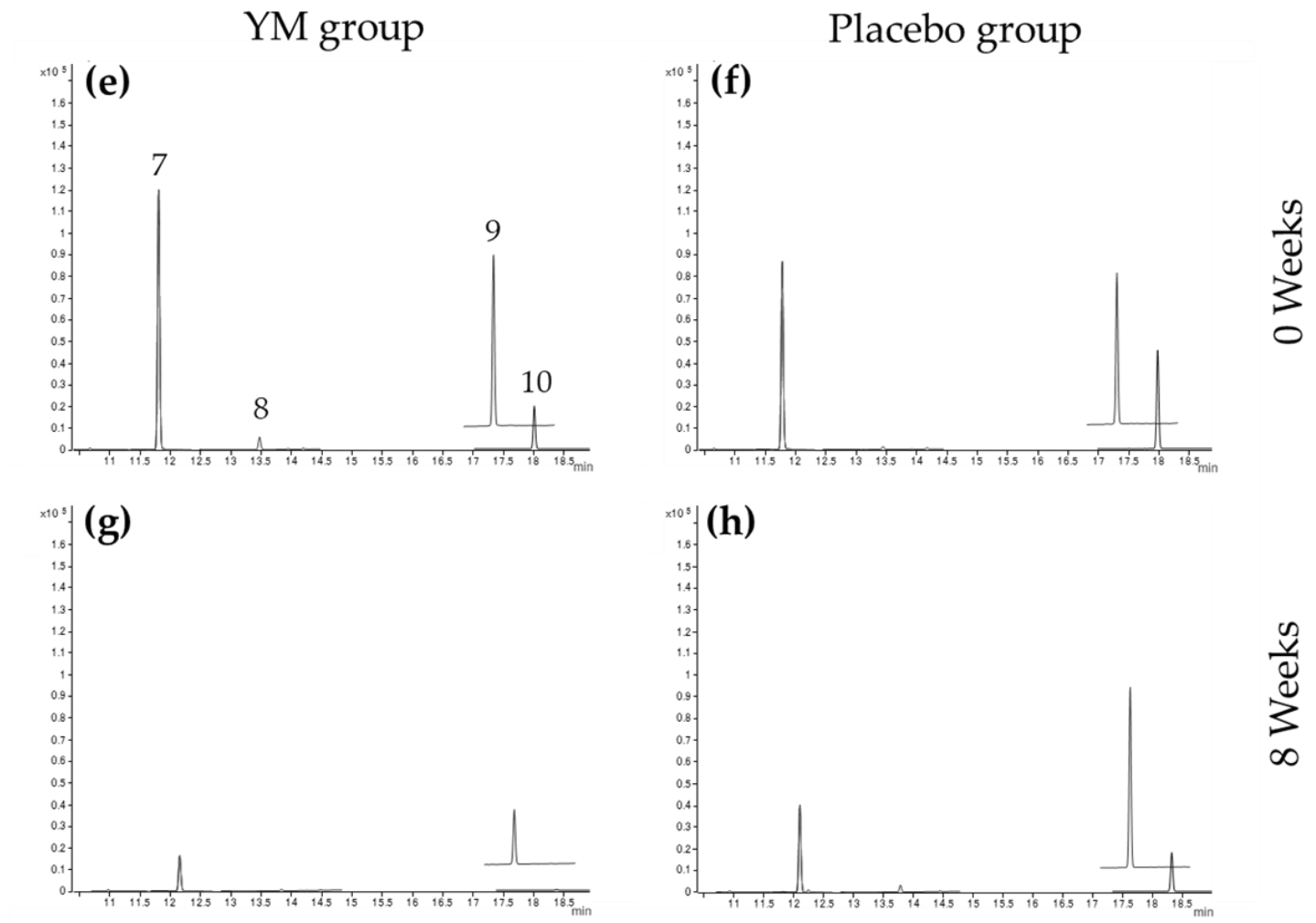


Figure S1. The typical chromatographic profiles of feces samples.

HPLC and GC-MS chromatograms in feces samples. HPLC chromatograms of YM group before intervention (a), HPLC chromatogram of Placebo group before intervention (b), HPLC chromatogram of YM group after 8 weeks intervention (c), HPLC chromatogram of Placebo group after 8 weeks intervention (d), GC-MS chromatogram of YM group before intervention (e), GC-MS chromatogram of Placebo group before intervention (f), GC-MS chromatogram of YM group after 8 weeks intervention (g), and GC-MS chromatogram of Placebo group after 8 weeks intervention (h): acetic acid (1), propionic acid (2), *iso*-butyric acid (3), *n*-butyric acid (4), *iso*-valeric acid (5), *n*-valeric acid (6), *p*-cresol (7), 4-ethylphenol (8), indole (9), skatole (10). HPLC analysis was performed using a Prominence organic acids analysis system with a CDD-10A conductivity detector (Shimadzu, Kyoto, Japan); two tandemly-arranged Shim-pack SCR-102(H) columns (300 mm × 8 mm inner diameter (i.d.)); a Shim-pack SCR-102(H) guard column (50 mm × 6 mm i.d.). 5 mM *p*-toluenesulfonic acid was used as a mobile phase and 5 mM *p*-toluenesulfonic acid with 100 μM EDTA and 20mM Bis-Tris was used as a pH buffering reagent. The flow rate was 0.8 mL/min. A column temperature was 45 °C. GC-MS analysis was performed using gas chromatography with mass spectrometry (5977A, Agilent Technologies, CA, USA) and a capillary column (DB WAX UI+G, 60 m × 0.25 mm × 0.25 μm, Agilent Technologies, CA, USA). Sample injection volume was 0.8 μl with splitless mode. Chromatography was performed under constant flow set at 1.7 ml/min. The GC oven was initially set to 70°C for 2 min after sample injection, then heated to 190°C at 25°C/min ramp rate, heated to 200°C at 2°C/min ramp rate, heated to 240°C at 25°C/min ramp rate, and kept constant at 240°C for 8 min. Mass spectra were recorded in the SIM mode. The ions monitored in SIM runs were: *m/z* 107.1 for *p*-cresol and 4-ethylphenol, *m/z* 117.1 for indole, and *m/z* 130.1 for skatole. SIM chromatograms were overlaid in a single GC-MS chromatogram chart. HPLC: High performance liquid chromatography. GC-MS: Gas chromatography and mass spectrometry. SIM: selective ion monitoring.