

In Vitro Fermentation of Different Indigestible Glucans with Varying Physico-Chemical Properties by Human Fecal Microbiota

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Contents

Figure S1. Changes of molecular weight of polysaccharides from indigestible glucans during fermentation.

Figure S2. Changes in the content of succinic acid during fermentation.

Figure S3. Bar chart for statistical analysis of pH and gas production.

Figure S4. Bar chart of gut microbiota composition.

Table S1. Changes in the content of lactic acid during fermentation.

Table S2. Released free monosaccharides and oligosaccharides during in vitro fermentation.

Table S3. Primer sequences used in real-time quantification PCR.

Table S4. Permutational MANOVA analysis of β -diversity among groups.

Method supplement

Changes of molecular weight of polysaccharides from indigestible glucans during fermentation.

Molecular weight of polysaccharide from indigestible glucans during fermentation was determined by high performance liquid chromatography (HPLC 1260, Agilent, USA) as described by Wang et al. [1], with modification. 100 μ L of supernatant (4 $^{\circ}$ C, 15 min, 13000 rpm) was mixed with 400 μ L of anhydrous ethanol and rested for 2 h to obtain sediment, the residual ethanol was removed by vacuum centrifugation, then re-dissolved with 1 mL of 0.02% NaN₃ mobile phase and filtered (0.22 μ m) for determination.

Standard curves were made using glucose and dextran (Dextran T-10, T-40, T-70, T-500, T-2000) as standards. Chromatographic conditions: Ultrahydrogel™ Linear (7.8 mm × 300 mm) column, 0.02% NaN₃ solution was used as mobile phase at a flow rate of 0.6 mL/min, column temperature of 35 °C, and data acquisition time was 30 min.

Result supplement

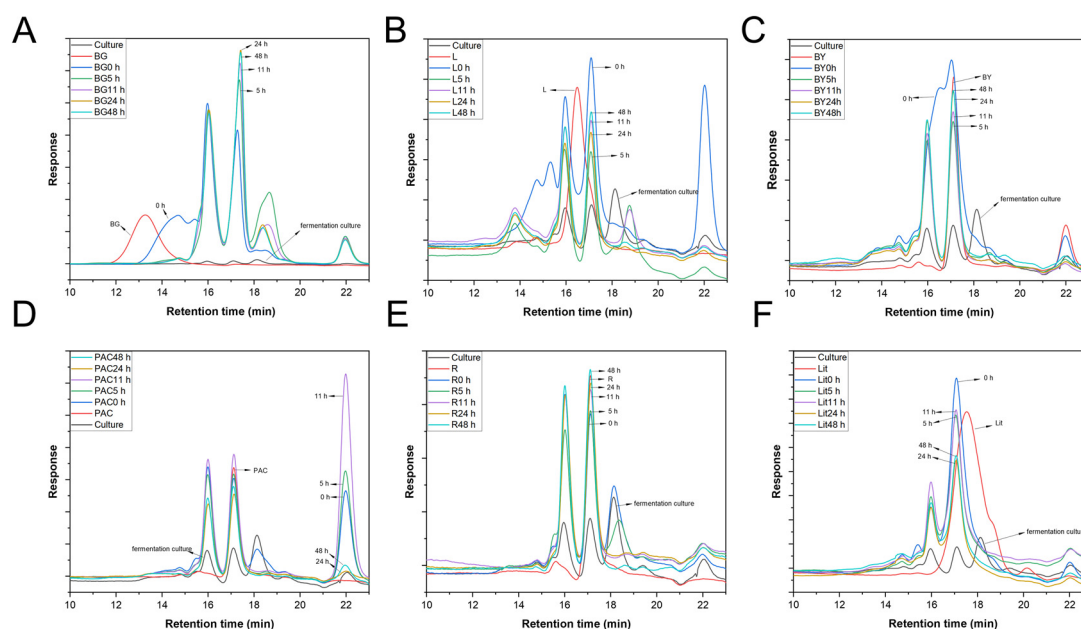


Figure S1. Changes of molecular weight of polysaccharides from indigestible glucans during fermentation. (A) Barley beta-glucan; (B) Laminarin; (C) Yeast beta-glucan; (D) Pachyman; (E) Resistant starch; (F) Litesse.

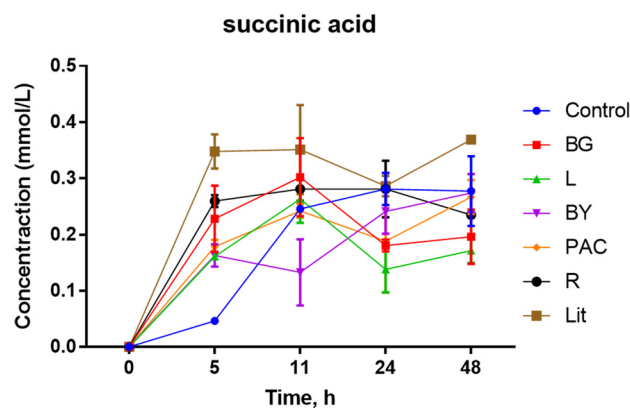


Figure S2. Changes in the content of succinic acid during fermentation.

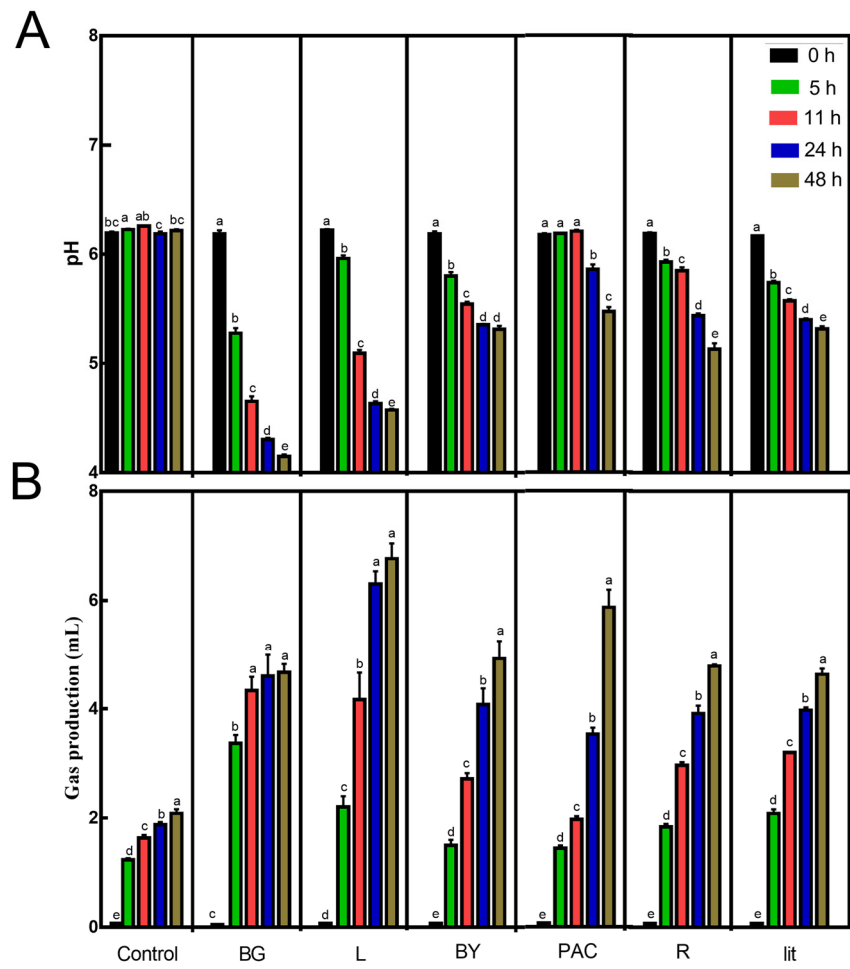


Figure S3. Bar chart for statistical analysis of pH and gas production. (A) pH change. (B) Gas production. BG, barley β -glucan; L, laminarin; BY, yeast β -glucan; PAC, pachyman; R, resistant starch; Lit, litesse. Different letters indicate significant difference between groups, $p < 0.05$.

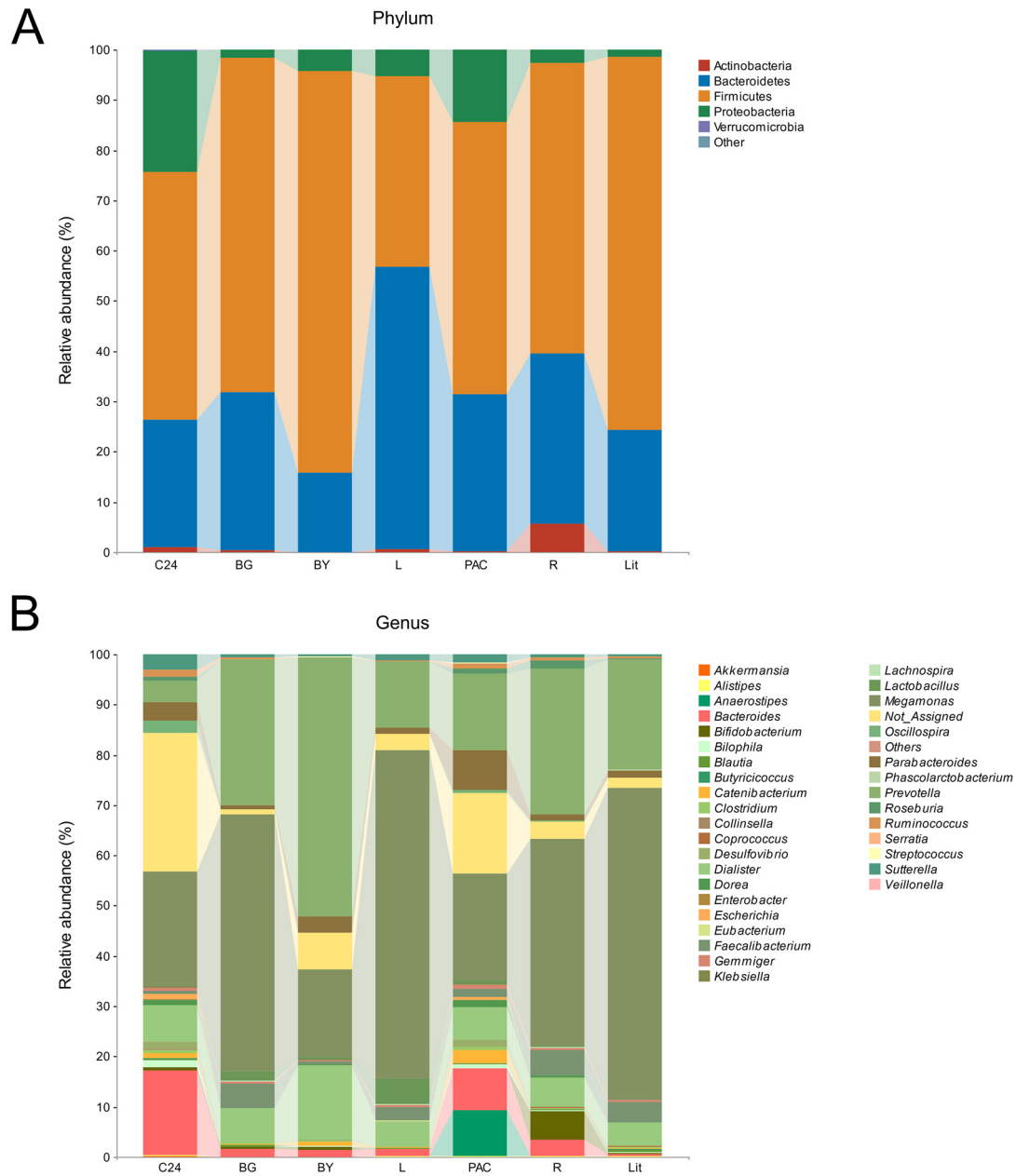


Figure S4. Bar chart of gut microbiota composition. (A) Phylum level;(B) Genus level. C24, control 24 h; BG, barley β -glucan; L, laminarin; BY, yeast β -glucan; PAC, pachyman; R, resistant starch; Lit, litesse.

Table S1. Changes in the content of lactic acid during fermentation.

| | Control | BG | L | BY | PAC | R | Lit |
|------|-------------------------------|-------------------------------|--------------------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|
| 0 h | 0.46 \pm 0 ^d | 1.26 \pm 0.07 ^c | 0.77 \pm 0.04 ^c | 1.26 \pm 0.13 ^b | 1.15 \pm 0.03 ^{ab} | 1.17 \pm 0.01 ^{cd} | 1.22 \pm 0 ^b |
| 5 h | 1.16 \pm 0.03 ^c | 1.23 \pm 0.05 ^c | 1.23 \pm 0.11 ^c | 1.37 \pm 0.1 ^b | 0.92 \pm 0.08 ^b | 1.1 \pm 0.01 ^d | 1.22 \pm 0.03 ^b |
| 11 h | 1.68 \pm 0.12 ^{ab} | 14.46 \pm 0.62 ^b | 11.91 \pm 0.18 ^a | 0.74 \pm 0.23 ^b | 1.29 \pm 0.09 ^{ab} | 1.77 \pm 0.04 ^{ab} | 1.30 \pm 0.53 ^b |
| 24 h | 1.81 \pm 0.20 ^a | 24.17 \pm 0.33 ^a | 8.85 \pm 1.88 ^b | 2.25 \pm 0.19 ^a | 1.87 \pm 0.48 ^a | 1.49 \pm 0.07 ^{bc} | 1.56 \pm 0.06 ^b |
| 48 h | 1.37 \pm 0.17 ^{bc} | 25.5 \pm 0.72 ^a | 10.89 \pm 0.84 ^{ab} | 2.5 \pm 0.47 ^a | 1.46 \pm 0.34 ^{ab} | 2.1 \pm 0.24 ^a | 2.42 \pm 0.20 ^a |

BG, barley β -glucan; L, laminarin; BY, yeast β -glucan; PAC, pachyman; R, resistant starch; Lit, litesse. Different letters indicate significant difference between groups, $p < 0.05$.

Table S2. Released free monosaccharides and oligosaccharides during in vitro fermentation (mg/mL).

| | Control | BG | L | BY | PAC | R | Lit |
|------------------------|-------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| Monosaccharide | | | | | | | |
| 0 h | ND | 10.16 ± 0.03 ^c | 0.4 ± 0.05 ^c | 0.25 ± 0.05 ^b | 0.11 ± 0.01 ^a | 0.29 ± 0.02 ^a | 0.36 ± 0.04 ^a |
| 5 h | ND | 3.10 ± 0.08 ^a | 2.19 ± 0.16 ^a | 0.5 ± 0.08 ^a | 0.01 ± 0 ^c | 0.06 ± 0.01 ^b | 0.56 ± 0.05 ^b |
| 11 h | ND | 1.20 ± 0.05 ^b | 1.24 ± 0.06 ^b | 0.24 ± 0.01 ^b | 0.02 ± 0.01 ^c | 0.11 ± 0.03 ^b | 0.32 ± 0.14 ^a |
| 24 h | ND | 0.08 ± 0.05 ^c | 0.05 ± 0.01 ^d | 0.04 ± 0.01 ^c | 0.07 ± 0.01 ^b | 0.06 ± 0.02 ^b | 0.14 ± 0.02 ^b |
| 48 h | 0.02 ± 0.01 | 0.10 ± 0.03 ^c | 0.01 ± 0 ^d | 0.01 ± 0.01 ^c | 0.05 ± 0.01 ^b | 0.06 ± 0.01 ^b | 0.09 ± 0.01 ^b |
| Disaccharide | | | | | | | |
| 0 h | ND | 0.02 ± 0 ^b | ND | 0.04 ± 0.02 ^a | ND | ND | *0±0 |
| 5 h | ND | 1.55 ± 0.05 ^a | 0.02 ± 0.01 ^a | ND | ND | ND | ND |
| 11 h | ND | 1.33 ± 0.05 ^a | *0 ± 0 ^b | ND | ND | ND | ND |
| 24 h | ND | 1.17 ± 0.41 ^a | ND | ND | ND | ND | ND |
| 48 h | ND | 1.45 ± 0.07 ^a | ND | ND | ND | ND | ND |
| Trisaccharide | | | | | | | |
| 0 h | ND | 0.02 ± 0 ^a | ND | 0.02 ± 0 ^a | ND | ND | *0 ± 0 ^b |
| 5 h | ND | 0.01 ± 0 ^b | 0.18 ± 0.06 ^a | *0 ± 0 ^b | ND | ND | 0.05 ± 0 ^a |
| 11 h | ND | *0 ± 0 ^b | 0.16 ± 0.01 ^a | ND | ND | ND | 0.02 ± 0.02 ^{ab} |
| 24 h | ND | *0 ± 0 ^b | *0 ± 0 ^b | ND | ND | ND | 0.01 ± 0.01 ^b |
| 48 h | ND | *0 ± 0 ^b | *0 ± 0 ^b | ND | ND | ND | 0.01 ± 0 ^b |
| Tetrasaccharide | | | | | | | |
| 0 h | ND | ND | ND | ND | ND | ND | ND |
| 5 h | ND | ND | ND | ND | ND | ND | ND |
| 11 h | ND | ND | ND | ND | ND | ND | ND |
| 24 h | ND | ND | ND | ND | ND | ND | 0.05±0 ^a |
| 48 h | ND | ND | ND | ND | ND | ND | 0.04±0 ^a |

¹Results are expressed as mean ± standard deviation ($n = 3$), and significant comparisons were made between groups of the same polysaccharide in the same column. Different letters indicate significant difference between groups, $p < 0.05$. ND indicates not detected by the equipment. * Indicates lower value, not shown (<0.01 mg/mL). BG, barley β -glucan; L, laminarin; BY, yeast β -glucan; PAC, pachyman; R, resistant starch; Lit, litesse.

Table S3. Primer sequences used in real-time quantification PCR.

| Target bacterium | Primer name | Sequence (5'-3') | Amplicon size (bp) | T _m (°C) | Reference |
|-------------------------------------|------------------------|---|--------------------|---------------------|--------------------|
| Total bacteria | 338F 518R | ACTCCTACGGGAGGCAGCAGT ATTACCGCGGCTGCTGGC | 192 | 60 | Castillo et al [2] |
| <i>Lactobacillus</i> | Lacto-F Lacto-R | GGAATCTTCCACAATGGACG CGCTTTACGCCCAATAAATCCGG | 217 | 56 | Bakar et al [3] |
| <i>Bifidobacterium</i> | Bifid-F Bifid-R | TCGCGTCYGGTGTGAAAG CCACATCCAGCRTCCAC | 243 | 58 | Rinttilä et al [4] |
| <i>Faecalibacterium prausnitzii</i> | FPR-2F Fprau645R | GGAGGAAGAAGGTCTTCGG AATTCCGCCTACCTCTGCACT | 245 | 60 | Carlett et al [5] |
| <i>Bacteroides</i> | g-Bfra-F g-Bfra-R | ATAGCCTTTTCGAAAGRAAGAT CCAGTATCAACTGCAATTTTA | 493 | 60 | Matsuki et al [6] |
| <i>Prevotella</i> | g-Prevo-F g-Prevo-R | CACRGTAACGATGGATGCC GGTCGGGTTGCAGACC | 515 | 56 | Matsuki et al [7] |

Table S4. Permutational MANOVA analysis of β -diversity among groups.

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | p-value | q-value |
|---------|---------|-------------|--------------|----------|---------|----------|
| BG24 | BY24 | 8 | 0.999 | 49.48552 | 0.026 | 0.040385 |
| BG24 | C0 | 8 | 0.999 | 68.27435 | 0.031 | 0.040385 |
| BG24 | C24 | 8 | 0.999 | 111.5937 | 0.028 | 0.040385 |
| BG24 | L24 | 8 | 0.999 | 9.321454 | 0.032 | 0.040385 |
| BG24 | Lit24 | 8 | 0.999 | 7.989927 | 0.034 | 0.040385 |
| BG24 | PAC24 | 8 | 0.999 | 64.91326 | 0.028 | 0.040385 |
| BG24 | R24 | 8 | 0.999 | 10.9191 | 0.036 | 0.0405 |
| BY24 | C0 | 8 | 0.999 | 24.86024 | 0.023 | 0.040385 |
| BY24 | C24 | 8 | 0.999 | 61.42028 | 0.035 | 0.040385 |
| BY24 | L24 | 8 | 0.999 | 51.91417 | 0.035 | 0.040385 |
| BY24 | Lit24 | 8 | 0.999 | 40.44521 | 0.028 | 0.040385 |
| BY24 | PAC24 | 8 | 0.999 | 39.01315 | 0.026 | 0.040385 |
| BY24 | R24 | 8 | 0.999 | 34.30895 | 0.034 | 0.040385 |
| C0 | C24 | 8 | 0.999 | 63.9431 | 0.017 | 0.040385 |
| C0 | L24 | 8 | 0.999 | 65.44541 | 0.03 | 0.040385 |
| C0 | Lit24 | 8 | 0.999 | 47.23702 | 0.028 | 0.040385 |
| C0 | PAC24 | 8 | 0.999 | 47.20966 | 0.017 | 0.040385 |
| C0 | R24 | 8 | 0.999 | 40.21948 | 0.03 | 0.040385 |
| C24 | L24 | 8 | 0.999 | 78.13104 | 0.034 | 0.040385 |
| C24 | Lit24 | 8 | 0.999 | 59.77891 | 0.032 | 0.040385 |
| C24 | PAC24 | 8 | 0.999 | 25.2231 | 0.029 | 0.040385 |
| C24 | R24 | 8 | 0.999 | 65.11511 | 0.029 | 0.040385 |
| L24 | Lit24 | 8 | 0.999 | 3.021266 | 0.053 | 0.056786 |
| L24 | PAC24 | 8 | 0.999 | 60.33565 | 0.026 | 0.040385 |
| L24 | R24 | 8 | 0.999 | 17.07078 | 0.034 | 0.040385 |
| Lit24 | PAC24 | 8 | 0.999 | 50.23807 | 0.027 | 0.040385 |
| Lit24 | R24 | 8 | 0.999 | 9.727374 | 0.028 | 0.040385 |
| PAC24 | R24 | 8 | 0.999 | 41.38957 | 0.028 | 0.040385 |

C0, control 0 h; C24, control 24 h; BG24, barley β -glucan 24 h; L24, laminarin 24 h; BY24, yeast β -glucan 24 h; PAC24, pachyman 24 h; R24, resistant starch 24 h; Lit24, litesse 24 h.

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