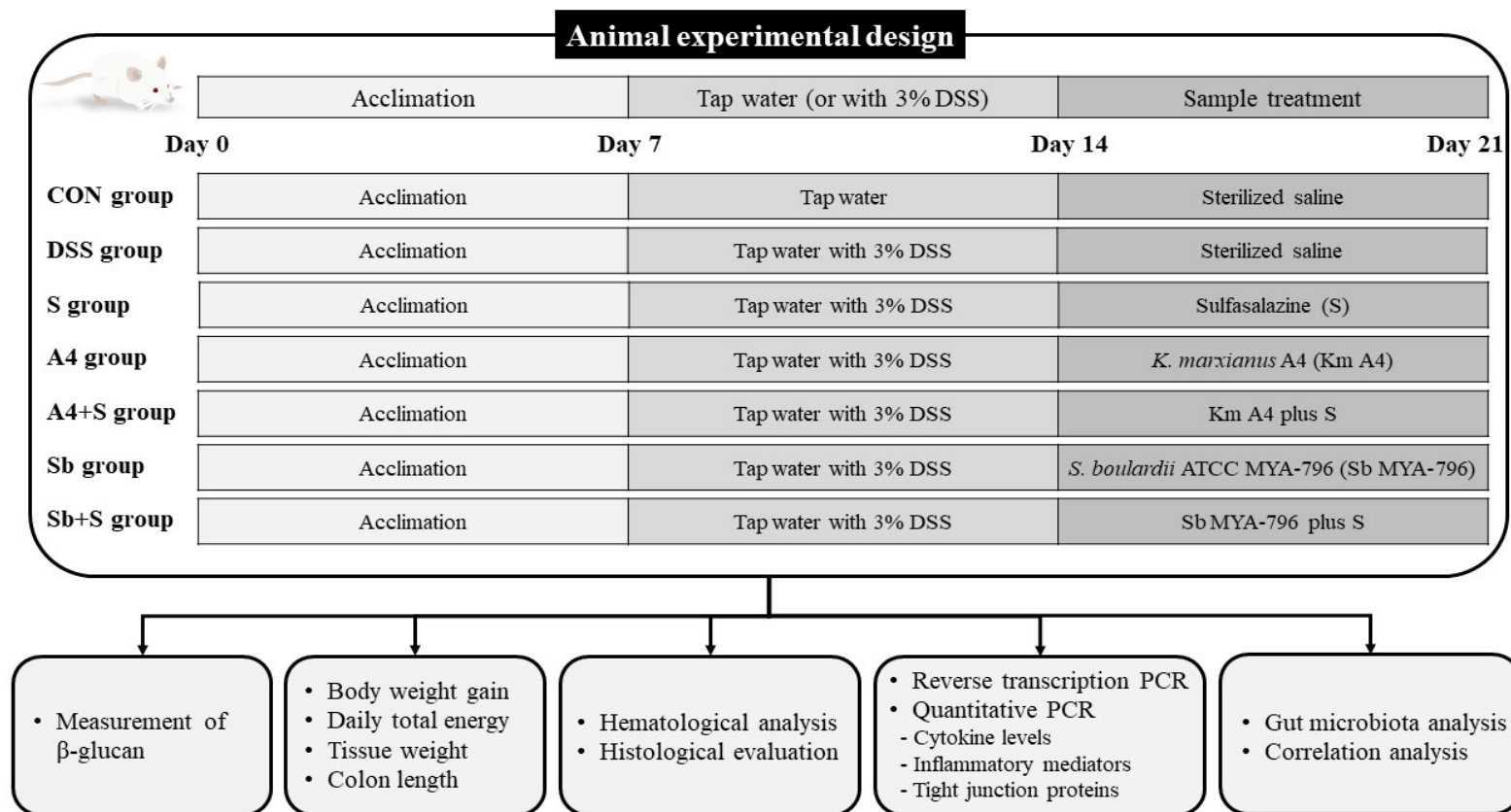


Electronic Supplementary Information



ESI file 1. Experimental framework for evaluating the anti-inflammatory effects of combined treatment with *Kluyveromyces marxianus* A4 and sulfasalazine compared to that of *Saccharomyces boulardii* ATCC MYA-796 in mice with dextran sulfate sodium (DSS)-induced colitis. **CON**, control (treated with sterilized saline after providing

autoclaved tap water); **DSS**, DSS-induced model (treated with sterilized saline after providing 3% DSS); **S**, sulfasalazine [treated with sulfasalazine (50 mg/kg/day) after providing 3% DSS]; **A4**, *K. marxianus* A4 (treated with 1×10^8 CFU/mL of *K. marxianus* A4 after providing 3% DSS); **A4+S**, *K. marxianus* A4 plus sulfasalazine [treated with 1×10^8 CFU/mL of *K. marxianus* A4 plus sulfasalazine (50 mg/kg/day) after providing 3% DSS]; **Sb**, *S. boulardii* ATCC MYA-796 (treated with 1×10^8 CFU/mL of *S. boulardii* ATCC MYA-796 after providing 3% DSS); **Sb+S**, *S. boulardii* ATCC MYA-796 plus sulfasalazine [treated with 1×10^8 CFU/mL of *S. boulardii* ATCC MYA-796 plus sulfasalazine (50 mg/kg/day) after providing 3% DSS].

ESI File 2. Scores for the histological evaluation of colitis.

Feature	Score	Description	Reference
Inflammation severity	0	None	
	1	Mild	
	2	Moderate	
	3	Severe	
Inflammation extent	0	None	
	1	Mucosa	
	2	Mucosa and submucosa	
	3	Transmural	
Crypt damage	0	None	1
	1	Basal 1/3 damaged	
	2	Basal 2/3 damaged	
	3	Crypts lost; surface epithelium present	
	4	Crypts and surface epithelium lost	
Percent involvement (%)	0	0	
	1	1–25	
	2	26–50	
	3	51–75	
	4	76–100	

ESI File 3. List of primers used to evaluate gene expression.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Accession number
<i>Gapdh</i>	CACTCACGGCAAATTCAACGGCAC	GACTCCACGACATACTCAGCAC	NM_008084.3
<i>Il-1β</i>	TCCAGGATGAGGACATGAGCAC	GAACGTCACACACCAGCAGGTTA	NM_008361.4
<i>Il-6</i>	TGACAACCACGGCCTTC	TTCTGCAAGTGCATCATCG	NM_031168.2
<i>Tnf-α</i>	AAGCCTGTAGCCACGTCGTA	GGCACCACTAGTTGGTTGTCTTTG	NM_013693.3
<i>Il-12p40</i>	CAGAAGCTAACCATCTCCTGGTTTG	CCGGAGTAATTTGGTGCTCCACAC	NM_001303244.1
<i>Ifn-γ</i>	GAAAGCCTAGAAAGTCTGAATAACT	ATCAGCAGCGACTCCTTTCCGCTT	NM_008337.4
<i>Il-10</i>	AGAAGCATGGCCAGAAATC	CCAAGGAGTTGTTCCGTTAGC	NM_010548.2
<i>Il-4</i>	AATGTACCAGGAGCCATATCCAC	TCACTCTGTGGTGTCTTCGT	NM_021283.2
<i>Cox-2</i>	CCGTGGTGAATGTATGAGCA	CCTCGCTTCTGATCTGTCTT	AY157736.1
<i>inos</i>	CTGCAGCACTTGGATCAGGAACCTG	GGGAGTAGCCTGTGTGCACCTGGAA	NM_010927.4
<i>Zo-1</i>	TGGAATTGCAATCTCTGGTG	CTGGCCCTCCTTTAACACA	NM_009386.2
<i>Ocln</i>	GCTGTGATGTGTGTGAGCTG	GACGGTCTACCTGGAGGAAC	U49185.1
<i>Cldn-1</i>	GACTGTTGATGATGGTTATCGG	AGATGGTAAGGTACAGCCAAGG	NM_016674.4

Gapdh, glyceraldehyde 3-phosphate dehydrogenase; **Il**, interleukin; **Tnf-α**, tumor necrosis factor-α; **Ifn-γ**, interferon-γ; **Cox-2**, cyclooxygenase-2; **inos**, inducible nitric

oxide synthase; **Zo-1**, zonula occludens-1; **Ocln**, occludin; **Cldn-1**, claudin-1.

ESI File 4. Body weight gain of the mice with dextran sulfate sodium (DSS)-induced colitis treated with sterilized saline, sulfasalazine, *Kluyveromyces marxianus* A4 (Km A4), Km A4 plus sulfasalazine, *Saccharomyces boulardii* ATCC MYA-796 (Sb MYA-796), and Sb MYA-796 plus sulfasalazine.

Groups	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
CON	0.0 ± 0.0 ^a	0.01 ± 0.12 ^a	0.20 ± 0.15 ^a	0.65 ± 0.09 ^a	0.83 ± 0.14 ^a	1.09 ± 0.16 ^a	1.53 ± 0.16 ^a	1.58 ± 0.10 ^a	1.98 ± 0.10 ^a	1.35 ± 0.19 ^a	1.48 ± 0.18 ^a	1.40 ± 0.19 ^a	1.56 ± 0.16 ^a	1.64 ± 0.19 ^a	1.86 ± 0.18 ^a
DSS	0.0 ± 0.0 ^a	0.35 ± 0.14 ^a	0.38 ± 0.18 ^a	1.11 ± 0.20 ^a	0.85 ± 0.21 ^a	0.70 ± 0.17 ^a	0.04 ± 0.11 ^a	-1.50 ± 0.21 ^b	-2.84 ± 0.27 ^b	-3.65 ± 0.28 ^b	-4.29 ± 0.41 ^c	-3.19 ± 0.70 ^b	-2.24 ± 0.77 ^b	-1.88 ± 0.70 ^b	-1.09 ± 0.62 ^b
S	0.0 ± 0.0 ^a	0.13 ± 0.08 ^a	0.51 ± 0.14 ^a	1.09 ± 0.18 ^a	0.99 ± 0.17 ^a	0.86 ± 0.19 ^a	0.24 ± 0.17 ^a	-1.16 ± 0.13 ^b	-2.38 ± 0.52 ^b	-2.93 ± 0.44 ^b	-3.10 ± 0.70 ^{bc}	-1.89 ± 0.76 ^b	-1.18 ± 0.83 ^{ab}	-0.90 ± 0.64 ^b	-0.31 ± 0.51 ^{ab}
A4	0.0 ± 0.0 ^a	0.14 ± 0.12 ^a	0.39 ± 0.10 ^a	0.86 ± 0.23 ^a	0.85 ± 0.23 ^a	0.81 ± 0.28 ^a	0.24 ± 0.38 ^a	-0.86 ± 0.47 ^b	-2.14 ± 0.42 ^b	-3.00 ± 0.40 ^b	-2.86 ± 0.55 ^{bc}	-1.38 ± 0.51 ^b	-0.39 ± 0.44 ^{ab}	-0.20 ± 0.52 ^{ab}	0.53 ± 0.40 ^{ab}
A4+S	0.0 ± 0.0 ^a	0.25 ± 0.15 ^a	0.60 ± 0.11 ^a	0.90 ± 0.12 ^a	0.82 ± 0.17 ^a	0.73 ± 0.33 ^a	-0.29 ± 0.38 ^a	-1.13 ± 0.43 ^b	-2.14 ± 0.21 ^b	-2.51 ± 0.20 ^b	-1.56 ± 0.41 ^b	-0.48 ± 0.50 ^{ab}	0.11 ± 0.31 ^{ab}	0.23 ± 0.24 ^{ab}	0.79 ± 0.16 ^{ab}
Sb	0.0 ± 0.0 ^a	0.09 ± 0.12 ^a	0.24 ± 0.18 ^a	0.76 ± 0.15 ^a	0.78 ± 0.22 ^a	0.43 ± 0.18 ^a	-0.24 ± 0.32 ^a	-1.51 ± 0.45 ^b	-2.56 ± 0.57 ^b	-3.39 ± 0.45 ^b	-3.50 ± 0.56 ^{bc}	-2.13 ± 0.55 ^b	-1.43 ± 0.64 ^b	-0.96 ± 0.58 ^b	-0.41 ± 0.52 ^{ab}
Sb+S	0.0 ± 0.0 ^a	0.10 ± 0.11 ^a	0.26 ± 0.15 ^a	0.91 ± 0.16 ^a	0.98 ± 0.15 ^a	0.69 ± 0.17 ^a	-0.05 ± 0.24 ^a	-1.39 ± 0.39 ^b	-2.41 ± 0.34 ^b	-2.94 ± 0.39 ^b	-2.96 ± 0.51 ^{bc}	-1.39 ± 0.52 ^b	-0.73 ± 0.46 ^{ab}	-0.14 ± 0.32 ^{ab}	0.41 ± 0.30 ^{ab}

All data are expressed as the mean ± standard deviation (n = 8). Different letters in a column indicate significant differences ($P < 0.05$), which were evaluated using a one-way analysis of variance followed by Tukey's test. **CON**, control (treated with sterilized saline after providing autoclaved tap water); **DSS**, DSS-induced model

(treated with sterilized saline after providing 3% DSS); **S**, sulfasalazine [treated with sulfasalazine (50 mg/kg/day) after providing 3% DSS]; **A4**, *K. marxianus* A4 (treated with 1×10^8 CFU/mL of *K. marxianus* A4 after providing 3% DSS); **A4+S**, *K. marxianus* A4 plus sulfasalazine [treated with 1×10^8 CFU/mL of *K. marxianus* A4 plus sulfasalazine (50 mg/kg/day) after providing 3% DSS]; **Sb**, *S. boulardii* ATCC MYA-796 (treated with 1×10^8 CFU/mL of *S. boulardii* ATCC MYA-796 after providing 3% DSS); **Sb+S**, *S. boulardii* ATCC MYA-796 plus sulfasalazine [treated with 1×10^8 CFU/mL of *S. boulardii* ATCC MYA-796 plus sulfasalazine (50 mg/kg/day) after providing 3% DSS].

ESI File 5. Significant relative taxonomy abundance ratio of the gut microbiota in mice with dextran sulfate sodium (DSS)-induced colitis treated with sterilized saline, sulfasalazine, *Kluyveromyces marxianus* A4 plus sulfasalazine, and *Saccharomyces boulardii* ATCC MYA-796 plus sulfasalazine at the phylum, class, order, family, and genus levels via 16S rDNA amplicon-based community analysis.

Biological taxonomy classification		Experimental groups				
		CON	DSS	S	A4+S	Sb+S
Phylum	Bacteroidota	81.98 ± 12.20 ^a	34.75 ± 6.50 ^b	54.06 ± 15.02 ^{ab}	74.63 ± 10.65 ^a	68.64 ± 17.41 ^{ab}
	Firmicutes	16.93 ± 12.08 ^b	63.35 ± 6.44 ^a	41.54 ± 14.01 ^{ab}	19.75 ± 15.53 ^b	29.95 ± 17.93 ^{ab}
Class	Bacteroidia	81.98 ± 12.20 ^a	34.75 ± 6.50 ^b	54.06 ± 15.02 ^{ab}	74.63 ± 10.65 ^a	68.64 ± 17.41 ^{ab}
	Clostridia	15.87 ± 12.25 ^b	60.87 ± 5.12 ^a	40.80 ± 13.11 ^{ab}	18.92 ± 16.04 ^b	29.61 ± 18.07 ^{ab}
Order	Bacteroidales	81.98 ± 12.20 ^a	34.74 ± 6.51 ^b	54.06 ± 15.02 ^{ab}	74.63 ± 10.65 ^a	68.64 ± 17.41 ^{ab}
	Oscillospirales	5.14 ± 5.55 ^b	18.53 ± 1.73 ^a	14.07 ± 0.86 ^{ab}	6.29 ± 6.28 ^{ab}	12.28 ± 5.58 ^{ab}
	Lachnospirales	7.33 ± 7.36 ^b	29.62 ± 2.87 ^a	16.67 ± 8.59 ^{ab}	8.50 ± 6.70 ^b	13.76 ± 11.05 ^{ab}
	Acholeplasmatales	0.00 ± 0.00 ^b	2.31 ± 2.41 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b
Family	Bacteroidaceae	60.62 ± 9.45 ^a	7.06 ± 1.25 ^c	24.47 ± 6.24 ^{bc}	46.98 ± 11.80 ^{ab}	42.93 ± 14.22 ^{ab}
	Rikenellaceae	6.60 ± 2.33 ^a	1.12 ± 0.97 ^b	2.57 ± 1.10 ^{ab}	3.60 ± 0.94 ^{ab}	3.69 ± 3.02 ^{ab}
	Oscillospiraceae	3.09 ± 2.82 ^b	13.59 ± 1.21 ^a	6.89 ± 0.80 ^b	3.63 ± 3.79 ^b	5.12 ± 1.88 ^b
	Lachnospiraceae	7.33 ± 7.36 ^b	29.62 ± 2.87 ^a	16.67 ± 8.59 ^{ab}	8.50 ± 6.70 ^b	13.76 ± 11.05 ^{ab}
Genus	<i>Bacteroides</i>	60.62 ± 9.45 ^a	7.06 ± 1.25 ^c	24.47 ± 6.24 ^{bc}	46.98 ± 11.80 ^{ab}	42.93 ± 14.22 ^{ab}
	<i>Alistipes</i>	6.60 ± 2.33 ^a	0.00 ± 0.00 ^b	2.12 ± 1.86 ^{ab}	3.60 ± 0.94 ^{ab}	3.11 ± 3.68 ^{ab}
	Unclassified	9.13 ± 3.08 ^b	20.98 ± 1.16 ^a	22.49 ± 4.27 ^a	16.08 ± 3.59 ^{ab}	19.24 ± 7.30 ^{ab}

Others	7.30 ± 3.20 ^b	21.88 ± 3.68 ^a	16.70 ± 5.00 ^a	9.10 ± 5.37 ^{ab}	9.79 ± 4.10 ^{ab}
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The relative taxonomy abundance ratio is expressed as a percentage and shown as the mean ± standard deviation (n = 3). Different letters in a row indicate significant differences ($P < 0.05$) using a one-way analysis of variance followed by Tukey's test or Kruskal–Wallis test followed by Dunn's test and Bonferroni correction. **CON**, control (treated with sterilized saline after providing autoclaved tap water); **DSS**, DSS-induced model (treated with sterilized saline after providing 3% DSS); **S**, sulfasalazine [treated with sulfasalazine (50 mg/kg/day) after providing 3% DSS]; **A4+S**, *K. marxianus* A4 plus sulfasalazine [treated with 1×10^8 CFU/mL of *K. marxianus* A4 plus sulfasalazine (50 mg/kg/day) after providing 3% DSS]; **Sb+S**, *S. boulardii* ATCC MYA-796 plus sulfasalazine [treated with 1×10^8 CFU/mL of *S. boulardii* ATCC MYA-796 plus sulfasalazine (50 mg/kg/day) after providing 3% DSS].

References

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