SUPPORTING INFORMATION

for

Fermented cereal-origin gerobiotic cocktails promote healthy longevity in *Caenorhabditis elegans*

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1. Resource Table

REAGENT/RESOURCE	SOURCES	IDENTIFIER
Chemicals, reagents, fluorescent probes, and kits		
5-Fluoro-2'-deoxyuridine	Sigma-Aldrich	F0503
Bile salt	Sigma-Aldrich	B8756
L-cysteine	Sigma-Aldrich	1.02838
2',7'-Dichlorodihydrofluorescein diacetate	Invitrogen	D399
ABTS	HiMedia	MB255
Acid Blue 9 (Blue food dye)	HiMedia	GRM366
Ascorbate	HiMedia	CMS040
Brain-heart infusion (BHI) agar	HiMedia	M211
DPPH	HiMedia	MB263
Ferrous sulphate	HiMedia	PCT0111
Gentamicin	HiMedia	TC026
Hydrochloric acid	HiMedia	TC536M
Hydrogen peroxide	HiMedia	PCT1511
Kanamycin	HiMedia	PCT1105
LB agar	HiMedia	M1151
Linoleic acid	HiMedia	GRM10250
MRS Agar	HiMedia	M641
MRS broth	HiMedia	GM369
Nicotinamide adenine dinucleotide	HiMedia	RM392
Nitro blue tetrazolium	HiMedia	PCT1209
Phenazine methosulfate	HiMedia	TC484
Potassium ferricyanide	HiMedia	GRM1034
Potassium nitrate	HiMedia	GRM1401
Potassium Nitrate	HiMedia	PCT0010
Sodium acetate	HiMedia	GRM410
Sodium azide	HiMedia	MB075
Sodium hydroxide	HiMedia	TC460
Streptomycin	HiMedia	PCT1120
Trichloroacetic acid	HiMedia	GRM6274
Trichloroacetic acid	HiMedia	MB293
Triton X-100	HiMedia	MB031
Xylene	HiMedia	AS080
β-carotene	HiMedia	RM2039
Culture media components	HiMedia	https://www.himedialabs.com/
RNeasy Mini Kit	Qiagen	74104
RevertAid First-strand cDNA synthesis kit	Thermo Scientific	K1622
TRIzol Reagent	Invitrogen	15596018
SYBR Green Master Mix	Sigma-Aldrich	KK4601

REAGENT/RESOURCE	SOURCES	IDENTIFIER
Experimental model: *C. elegans*		
Wild-type	CGC, MN	Bristol N2
daf-2(*e1370*)	CGC, MN	CB1370
age-1(*hx546*)	CGC, MN	TJ1052
daf-16(*mu86*)	CGC, MN	CF1038
skn-1(*zu67*)	CGC, MN	EU1
hsf-1(*sy441*)	CGC, MN	PS3551
jnk-1(*gk7*)	CGC, MN	VC8
mpk-1(*ku1*)	CGC, MN	MH37
pmk-1(*km25*)	CGC, MN	KU25
tir-1(*ok1052*)	CGC, MN	RB1085
nsy-1(*ag3*)	CGC, MN	AU3
sek-1(*km4*)	CGC, MN	KU4
sir-2.1(*ok434*)	CGC, MN	VC199
aak-2(*ok524*)	CGC, MN	RB754
eat-2(*ad1116*)	CGC, MN	DA1116
bar-1(*ga80*)	CGC, MN	EW15
dbl-1(*nk3*)	CGC, MN	NU3
muIs84(*sod-3::GFP*)	CGC, MN	CF1553
dvIs70(*hsp-16.2p::GFP*)	CGC, MN	CL2070
zIs356(*daf-16::GFP*)	CGC, MN	TJ356
ldIs7(*skn-1b/c::GFP*)	CGC, MN	LD1
dvIs19(*gst-4::GFP*)	CGC, MN	CL2166
ldIs3(*gcs-1::GFP*)	CGC, MN	LD1171
xnIs17(*dlg-1::GFP*)	CGC, MN	FT63
Bacterial strains		
Escherichia coli	CGC, MN	OP50
Pseudomonas aeruginosa	MTCC, India	424
Staphylococcus aureus	MTCC, India	3160
Lactobacillus rhamnosus GG	ATCC, VA	53103
Oligonucleotides		
daf-2 F: 5'-AAAAGATTTGGCTGGTCAGAGA-3' *daf-2* R: 5'-TTTCAGTACAAATGAGATTGTCAGC-3'		Obtained from bioserve (https://bioserve.in/)
daf-16 F: 5'-TTCAATGCAAGGAGCATTTG-3' *daf-16* R: 5'-AGCTGGAGAAACACGAGACG-3'		
skn-1 F: 5'-CTCTCTTCTGGCATCCTCTACCA-3' *skn-1* R: 5'-TTCTTGGATTCTTCTTCTTGTTCGT-3'		
sod-3 F: 5'-TTCAAAGGAGCTGATGGACACT-3' *sod-3* R: 5'-AAGTGGGACCATTCCTTCCAA-3'		
hsp-16.2 F: 5'-GATGCTCGTGCTCTTGCTG-3' *hsp-16.2* R: 5'-CCGAATTGTTCTCCATCGAC-3'		
gst-4 F: 5'-CTATTTCCGTCCAGCTCAAC-3' *gst-4* R: 5'-TTTGTTCAACGGGCGCTTGC-3'		
age-1 F: 5'-GCTGCTCCGTGCAGAGATTG-3' *age-1* R: 5'-CACGGAGGTAAGCTTCCATC-3'		

REAGENT/RESOURCE	SOURCES	IDENTIFIER
dbl-1 F: 5'-GCCATTCTCCACCTCTTCCT-3' *dbl-1* R: 5'-GGAACATCAATGCTCGGACC-3'		
bar-1 F: 5'-GACGAGATTCATCAAAACCATACAA-3' *bar-1* R: 5'-TACTCGGACCAGGGTTTGAT-3'		
tir-1 F: 5'-CCGACCACCAAAGAAATGCC-3' *tir-1* R: 5'-CTTGGTCCACCGATGCTTCT-3'		
pmk-1 F: 5'- ACTTCATCCGACTCCACGAG-3' *pmk-1* R: 5'- CAGCAGCACAAACAGTTCCA-3'		
sek-1 F: 5'-TGCTCAACGAGCTAGACG-3' *sek-1* R: 5'-ATGTTCGACGGTTTCACG-3'		
nsy-1 F: 5'-TGCGATGAACTACTACGG-3' *nsy-1* R: 5'-CACCCAAATGACCAAATA-3'		
hlh-1 F: 5'-GTCACCGCAAATGACATCAC-3' *hlh-1* R: 5'- GGTAGGTGCAGTTGGAGCAT-3'		
unc-120 F: 5'-*TGCCTTAATGCTCCTGGTGG*-3' *unc-120* R: 5'- *GGGGCGAGAAATGGAGTGAA*-3'		
act-1 F: 5'-GCTGGACGTGATCTTACTGATTACC-3' *act-1* R: 5'-GTAGCAGAGCTTCTCCTTGATGTC-3'		
Software and tools		
MedCalc v.14.8.1	MedCalc Software Ltd.	https://www.medcalc.org/
SPSS 16	IBM SPSS Statistics	https://www.ibm.com/products/spss-statistics
Excel 2016	Microsoft	https://www.microsoft.com/en-us/microsoft-365/excel
PowerPoint 2016	Microsoft	https://www.microsoft.com/en-us/microsoft-365/powerpoint
Optika ProView	OPTIKA Science	https://www.optikascience.com/optikascience/?lang=en
MEGA 11	Molecular Evolutionary Genetics Analysis	https://www.megasoftware.net/
BLAST	NCBI	https://blast.ncbi.nlm.nih.gov/Blast.cgi
GenBank	NCBI	https://www.ncbi.nlm.nih.gov/genbank/
ImageJ	NIH	https://imagej.nih.gov/ij/index.html

2. MATERIALS AND METHODS

2. 1. Isolation, genetic identification, and characterization of probiotic bacteria

Probiotic bacteria were isolated from traditionally fermented (natural and uncontrolled fermentation in clay pots) barnyard millet (*Echinochloa frumentacea*) samples. Briefly, about 10 g of fermented barnyard millet samples were homogeneously mixed with sterilized phosphate buffer saline (PBS; pH 6.8), and 1 mL of serially diluted samples were spread onto de Man Rogosa and Sharpe (MRS) agar plates. The plates were incubated at 37°C for 24-48 h, and the pure colonies were obtained after successive cultivation by the streak plate method. We performed Gram staining and catalase activity assay to exclude gram-negative and gas-forming bacteria, respectively, from selected isolates. The pure cultures were stored at -80°C in MRS broth containing 30% glycerol. Genus- and species-level identification of selected isolates was determined by sequencing the 16S rRNA gene. The 16S rRNA sequencing analysis was performed with universal primers (Primer_F-GAGTTTGATCGTGGCTCAG; Primer_R-AGGGCTACCTTGTTAGACTT). The resulting sequence data were aligned and analyzed using a basic local alignment search tool (BLAST, NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the identification was confirmed based on the highest hit scores. At last, the obtained sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/), and accession numbers were assigned. Lists of identified bacteria were summarized in the main text **Table 1**.

2.2. Tolerance to different pH and bile-salts

Tolerance to different pH and bile salts was evaluated according to the method described previously.[1] For low pH tolerance assay, the pH of freshly prepared MRS broth was adjusted from 2.0 to 7.0 using Hydrochloric acid (HCl) or sodium hydroxide (NaOH) and 100 µL of active cultures were resuspended in it for 3 h at 37°C. After incubation, bacterial suspensions were further serially diluted using phosphate buffer saline (PBS; pH 7.2), spread onto MRS agar plates and incubated for 48 h at 37°C. Total cell viability was enumerated by colony counting method. For the bile-salt tolerance assay, 100 µL of an overnight-grown probiotic strains were inoculated into MRS broth supplemented with 0.1, 0.2, and 0.3% (w/v) bile (Oxgall, Sigma) and incubated at 37°C for 4 h. After incubation, 50 µL of serially diluted bacterial suspension were spread onto MRS agar plates and incubated for 48 h. Total cell viability was enumerated by colony counting method. Simultaneously, low pH and bile-salt tolerance of isolated probiotic

strains were also determined by measuring the bacterial growth at 580 and 620 nm, respectively, using a UV-Vis spectrophotometer.

2.3. Auto- and co-aggregation abilities

The specific cell-cell interactions were determined using the auto- and co-aggregation assay according to the methodology described by Kos et al.[2] Overnight culture of selected probiotic strain was centrifuged at 10000 rpm for 10 min, washed twice with PBS (pH 7.2) and re-suspended in PBS to a to 10^{-8} CFU/mL. The bacterial suspension was mixed by gentle vortexing for 20 s, and absorbance was measured at 600 nm (A_0) using a UV-Vis spectrophotometer. The suspension was then incubated at 37°C for 3 h, and the absorbance of the supernatant was measured at 600 nm (A_t). The percentage auto-aggregation was calculated as per the following formula:

$$Autoaggregation\ (\%) = \left(\frac{(A_0 - A_T)}{A_0}\right) \times 100$$

To examine the co-aggregation between selected isolates of probiotics and pathogens (either *Pseudomonas aeruginosa* [PA] or *Staphylococcus aureus* [SA]), an equal volume of cell suspension (1.5 mL) was mixed in pair by vortexing for 20 s, incubated for 3 h at 37°C and the absorbance of supernatant was measured at 600 nm. The percentage co-aggregation was calculated as per the following formula:

$$Coaggregation\ (\%) = \left(\frac{(A_{PAT} + A_{LAB})/(2 - A_{MIX})}{(A_{PAT} + A_{LAB})/2}\right) \times 100$$

where the factors A_{PAT} and A_{LAB} represent the absorbance of the individual pathogen and LAB, respectively. A_{MIX} represents the absorbance of mixed bacterial suspensions after incubation. Each assay was performed at least three times for each LAB strain under similar conditions, and each experiment contained five replicates.

2.4. Cell surface hydrophobicity

The adhesion of LAB to xylene was used to determine its cell surface hydrophobicity [3]. First, stationary phase cells from LAB strains were collected by centrifugation, dissolved in 3 mL of 0.1 M KNO_3, and the absorbance at 600 nm was determined ($A_{Control}$). Next, an aliquot of xylene (1 mL) was added to the cell suspension and incubated at room temperature for 10 minutes to establish phase separation (an organic phase and an aqueous phase). After vortexing,

the tubes were incubated again at room temperature for 20 minutes, and the absorbance of the aqueous phase was recorded at 600 nm (A_{Test}). Finally, the percent cell surface hydrophobicity (CSH) was calculated using the following equation:

$$CSH\ (\%) = \left(\frac{A_{Control} - A_{Test}}{A_{Control}}\right) \times 100$$

Three independent biological experiments were performed, and each biological sample was analyzed with five technical replicates.

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity assay

To examine the *in vitro* antioxidant properties of selected probiotic strains, 2, 2-diphenyl-1-picrylhydrazyl (DPPH, HiMedia) free radical quenching experiment was performed. The reaction mixture consisted of 2 mL of each probiotic strain (10^8 CFU/mL) and 2 mL of DPPH solution (0.1 mM DPPH in methanol) was mixed vigorously and then the tubes were incubated for 20 min in the dark at room temperature. After incubation, decolorization of the reaction mixture was measured at 517 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). The percentage inhibition of DPPH radicals (IC_{50}) by probiotic strains was calculated using the following equation, and comparable with ascorbic acid.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \left(1 - \frac{A_1}{A_2}\right) \times 100$$

Where A_1 and A_2 represent the absorbance of the samples and control (distilled water), respectively, mixed with DPPH solution.

2.5.2. Superoxide scavenging activity assay

The superoxide anion scavenging activity of probiotics was determined according to the method previously described.[4] Initially, the reaction mixture was prepared by mixing 0.2 M sodium phosphate buffer, 15 µM phenazine methosulfate, 50 µM nitrobluetetrazolium, and 75 µM nicotinamide adenine dinucleotide (NADH). 1 mL of reaction mixture was added to 50 µL of LAB strains and incubated at 37°C for 10 minutes. The absorbance of the samples at 560 nm was measured and the scavenging activity was calculated as,

$$Superoxide\ anion\ scavenging\ activity\ (\%) = \left(\frac{A_1 - A_2}{A_1}\right) \times 100$$

Where A_1 and A_2 represent the absorbance of the control and sample, respectively. Three independent biological experiments were performed, and each biological sample was analyzed with five technical replicates.

2.5.3. Reducing power activity

Reducing power activity of probiotic strains isolated from fermented barnyard miller was determined according to the method described previously.[5] Briefly, 0.2 mL of cell suspension was mixed with 200 µL of 1% potassium ferricyanide and 200 µL of 200 mM sodium phosphate buffer (pH 7.2), and the mixture was incubated for 20 min at 50°C. After incubation, the reaction mixture was centrifuged at 10,000 rpm at 4°C after adding 200 µL of 10% (w/v) trichloroacetic acid. The supernatant (500 µL) was mixed with 100 µL of 0.1% ferric chloride and 400 µL of distilled water. After 10 min incubation, the absorbance was read at 700 nm, and comparable with L-Cysteine.

2.5.4. Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity of isolated probiotic bacteria was conducted using the method of Ding et al. (2017).[6] The reaction mixture was prepared as follows: 1 mL of O-phenanthroline (0.1% w/v), 1 mL of phosphate buffer saline, 1 mL of 2.5 mM ferrous sulphate (Fe_2SO_4), and 1 mL of 20 mM hydrogen peroxide (H_2O_2). Cell suspension (500 µL) was mixed with reaction solution, incubated at 37°C for 2 h, and the absorbance was measured at 536 nm. The percentage of hydroxyl radical scavenging activity was calculated using the following equation;

$$Scavenging\ activity\ (\%) = \left(\frac{A_1 - A_2}{A_1 - A_0}\right) \times 100$$

Where A_1 is the absorbance in the presence of H_2O_2 alone, A_2 is the absorbance in the presence of sample and H_2O_2 and A_0 is the absorbance of blank (in the absence of sample and H_2O_2).

2.5.5. 2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity

The ABTS radical scavenging assay was performed using 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) as described.[7] The ABTS cation (ABTS+) was produced by reacting 7 mM ABTS stock solution with 5.5 mM potassium persulfate to stand in the dark for at least 6 h before use. The ABTS+ solution was diluted with distilled water to an

adjusted absorbance of 0.8 at 734 nm before use. Bacterial strains (150 µL) were mixed with 1.35 mL of ABTS+ solution, incubated at 37°C for 10 min, and the absorbance at 734 nm was measured. The ABTS radical scavenging activity was calculated using the following formula:

$$ABTS\ radical\ scavenging\ activity\ (\%) = \left(1 - \frac{A_1}{A_2}\right) \times 100$$

Where A_1 and A_2 represent the absorbance of the samples and control (distilled water), respectively, mixed with ABTS+ solution. Three independent biological experiments were performed, and each biological sample was analyzed with five technical replicates.

2.5.6. β- Carotene bleaching inhibitory activity

For the β-carotene bleaching inhibitory activity assay, the reaction mixture (β-carotene-linoleic acid) was prepared by adding 3 mg β-carotene, 66 µL linoleic acid, 300 µL Tween 80, and 10 mL chloroform. The reaction mixture was then evaporated using a vacuum evaporator and diluted with distilled water (75 mL). Subsequently, 4 mL of reaction mixture were mixed with 200 µL of LAB strains and incubated at 37°C for 2 h in the dark. The absorbance of the samples at 470 nm was measured and percentage inhibition was calculated as,

$$\beta\text{-}Carotene\ bleaching\ inhibitory\ activity\ (\%) = \left(\frac{A_{1,\ 2h} - A_{2,\ 2h}}{A_{2,\ 0h} - A_{2,\ 2h}}\right) \times 100$$

where A_1 and A_2 represent the absorbance of samples and control (distilled water), respectively. Three independent biological experiments were performed, and each biological sample was analyzed with five technical replicates

2.5.7. Inhibition of ascorbate autoxidation

The cell-free extract of LAB strains (0.1 mL from 10^8 CFU mL^{-1}) or distilled water (control) was mixed with 9.8 ml of 0.1 mL of 5 mmol/L ascorbate solution and 0.2 mol/L sodium phosphate buffer (pH 7.0). The reaction mixture was incubated at 37°C for 10 minutes. After incubation, 3 mL reaction mixture was then transferred to a cuvette and the absorbance at 265 nm was measured. Inhibition of ascorbate autoxidation was calculated as,

$$Inhibition\ rate\ (\%) = \left(\frac{A_1 - A_2}{A_1}\right) \times 100$$

Where A_1 and A_2 represent the absorbance of the control and sample, respectively. Three independent biological experiments were performed, and each biological sample was analyzed with five technical replicates.

2.6. Anti-pathogenic activity

The anti-pathogenic activity of selected probiotic strains was tested against three human pathogens *viz*., *Pseudomonas aeruginosa* - 422 MTCC and *Staphylococcus aureus* -96 MTCC (procured from Institute of Microbial Technology, Chandigarh, India) using agar well diffusion method. Each bacterial pathogen was spread on Muller-Hinton Agar (MHA) medium and 6 mm in diameter wells were made on plates using a cork borer. Cultures of selected probiotic strains after 24 h cultivation in MRS broth were centrifuged at 10,000 rpm for 10 min, and 100 µL of each supernatant was added into the wells made on MHA agar plates. The plates were allowed to dry, incubated at 37°C overnight, and the diameters (mm) of the clear inhibition zone were measured.

2.7. Body bends

Control and probiotic-fed worms were washed gently with M9 buffer and released on the unseeded agar plates to crawl for 5 minutes at 20°C. We then transferred individual worms (n=20 worms per experiment) to a 24-well tissue culture plate containing 1 mL M9 buffer. After 1 min recovery period, the number of body bends was scored for 30 seconds using an inverted stereo zoom microscope. The reciprocating motion of bending at the mid-body of *C. elegans* was considered a body bend.[8]

2.8. Chemotaxis behavior

Chemotaxis assay was performed according to the Bargmann et al. method.[9] Briefly, the age-synchronized worms (~100 worms per treatment) were raised on plates seeded with *E. coli* OP50 or LAB strains. Day 12 adulthood stage worms were transferred to chemotaxis plates (90 mm) divided into four equal quadrants (A_1, B_1, A_2, B_2) carrying 10 µL attractant (1 M sodium acetate) on one side (A1, A_2) and 10 µL distilled water on the other side (B_1, B_2). Sodium azide (25 mM, 20 µL) was spotted on each side to paralyze the attracted worms towards the region. The worms were released at the center of plates and incubated at 20°C for 90 min, and the chemotaxis index (CI) was calculated using the following formula;

$$CI = \frac{(A_1 + A_2) - (B_1 + B_2)}{N}$$

Where A_1 and A_2 represent worms in the attractant region, B_1 and B_2 represent worms in the control region, and N represents the total number of worms. Three independent biological experiments were performed, and each biological sample was analyzed with three technical replicates.

2.9. Gut Health Assay

Gut health was analyzed by measuring intestinal barrier functions using blue food dye and GFP-labelled transgenic strain FT63. Wild-type worms were raised as described above in the lifespan assay. At indicated time points, worms were removed from the treatment plates and suspended for 3 h in liquid NGM containing *E. coli* OP50 mixed with blue food dye (FD&C Blue #1/Acid Blue 9; 5 % wt/v). Worms were then washed with M9 buffer, mounted on to agar padded microscopic slides, and analyzed for the presence or absence of blue food dye in the body cavity.[10] The gut barrier integrity was further analyzed using the transgenic strain FT63 (*dlg-1::GFP*) expressing GFP in the epithelial cell junctions. Worms carrying the *dlg-1::GFP* transgene were continuously fed *E. coli* OP50 or LAB, as indicated above. Animals were then visualized under a fluorescence microscope (BX41, Olympus, Japan), and GFP signals from epithelial cells were imaged. Intact gut integrity is represented by clearly ordered bamboo-like GFP fluorescence, and disorganization (or even disappearance) of an orderly-edged GFP fluorescence indicates loss of gut integrity. For each time point, three independent experiments were performed, each with 10-15 worms per replicate.

3. References

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Table S1. Preliminary screening showing the *in vitro* probiotic attributes (Gram staining and gas formation) of isolated probiotic strains from fermented barnyard millet

S. No.	Isolates	Probiotics	Gram staining	Catalase activity
1	PS4	*Lactococcus lactis*	+	-
2	PS5	*Lactococcus lactis*	+	-
3	PS7	*Enterococcus faecium*	+	-
4	PS8	*Weissella confusa*	+	-
5	PS9	*Lactococcus lactis*	+	-
6	PS10	*Lactococcus lactis*	+	-
7	PS16	*Weizmannia coagulans*	+	-
8	PS19	*Weizmannia coagulans*	+	-
9	PS20	*Weizmannia coagulans*	+	-
10	PS21	*Enterococcus faecium*	+	-
11	PS22	*Weizmannia coagulans*	+	-
12	PS24	*Weizmannia coagulans*	+	-
13	PS25	*Lacticaseibacillus rhamnosus*	+	-
14	PS26	*Lacticaseibacillus rhamnosus*	+	-
15	PS27	*Enterococcus italicus*	+	-
16	PS28	*Lacticaseibacillus rhamnosus*	+	-
17	PS30	*Weizmannia coagulans*	+	-
18	PS31	*Lacticaseibacillus rhamnosus*	+	-
19	PS32	*Lacticaseibacillus rhamnosus*	+	-
20	PS40	*Limosilactobacillus fermentum*	+	-
21	PS46	*Lactiplantibacillus plantarum*	+	-
22	PS47	*Weissella confusa*	+	-
23	PS48	*Weissella confusa*	+	-
24	PS49	*Lactobacillus amylovorus*	+	-
25	PS52	*Limosilactobacillus fermentum*	+	-
26	PS55	*Lactobacillus amylovorus*	+	-
27	PS56	*Enterococcus italicus*	+	-
28	PS57	*Lactiplantibacillus plantarum*	+	-
29	PS60	*Lactobacillus amylovorus*	+	-
30	PS68	*Lactobacillus amylovorus*	+	-
31	PS69	*Lactobacillus delbrueckii* subsp. *bulgaricus*	+	-
32	PS70	*Bacillus licheniformis*	+	-
33	PS74	*Bacillus licheniformis*	+	-
34	PS75	*Bacillus licheniformis*	+	-

35	PS77	*Lactobacillus delbrueckii* subsp. *bulgaricus*	+	-
36	PS78	*Lactobacillus delbrueckii* subsp. *bulgaricus*	+	-
37	PS79	*Lactobacillus delbrueckii* subsp. *bulgaricus*	+	-
38	PS82	*Pediococcus pentosaceus*	+	-
39	PS91	*Pediococcus pentosaceus*	+	-

Note: +, positive; -, negative

Table S2. Preliminary screening showing the *in vitro* probiotic attributes (tolerance to acid and bile salts) of isolated probiotic strains from fermented barnyard millet

S. No.	Isolates	Acid tolerance (%)			Bile tolerance (%)		
		pH 5	pH 4	pH 3	0.1%	0.2%	0.3%
1	PS4	63.7±1.0	56.4±2.6	52.1±3.6	37.8±1.6	33.9±2.3	31.3±3.6
2	PS5	65.8±4.0	63.8±3.2	57.2±2.1	30.7±2.2	30.1±3.4	28.9±2.1
3	PS7	48.4±3.5	45.6±2.6	42.9±0.6	52.2±3.1	49.2±4.2	48.6±1.6
4	PS8	55.4±1.0	51.8±5.5	47.0±1.6	44.6±1.6	44.3±2.3	41.7±0.5
5	PS9	42.9±2.6	38.4±2.6	34.9±0.6	26.0±0.5	24.9±2.6	23.5±1.6
6	PS10	95.8±3.6	87.4±3.2	82.0±0.6	92.3±2.1	89.5±2.7	85.4±2.3
7	PS16	49.5±1.5	46.5±4.5	43.7±2.6	24.5±1.6	23.1±3.1	22.3±1.9
8	PS19	56.3±1.9	51.1±2.8	49.3±2.1	51.0±0.5	59.4±3.7	58.4±2.0
9	PS20	41.1±2.4	39.8±5.5	37.6±1.6	41.1±2.6	40.0±3.6	38.7±2.1
10	PS21	32.2±4.6	27.8±3.1	24.6±1.7	36.8±3.1	34.2±1.4	31.9±3.0
11	PS22	49.7±3.2	46.4±2.0	44.3±1.0	40.4±2.6	48.8±3.6	47.6±1.6
12	PS24	22.9±0.5	22.1±1.8	21.3±3.0	38.0±0.5	34.4±2.4	33.4±2.3
13	PS25	46.1±2.5	45.0±0.6	44.2±1.2	52.0±2.5	50.4±0.8	59.3±1.6
14	PS26	59.1±2.5	58.1±1.6	57.7±2.5	39.7±2.8	38.6±1.6	38.1±2.3
15	PS27	40.8±2.8	38.2±0.6	37.7±2.8	42.1±3.6	49.5±0.9	48.1±3.4
16	PS28	95.0±1.0	91.1±3.2	87.9±2.4	94.7±1.6	92.7±1.6	83.5±3.6
17	PS30	23.0±1.1	22.6±2.6	21.0±2.1	27.2±1.8	24.7±0.6	23.2±3.7
18	PS31	38.8±1.6	37.1±2.9	35.7±0.6	38.7±2.6	35.5±1.4	33.7±3.4
19	PS32	44.8±2.3	44.3±5.4	43.5±1.6	41.3±3.6	39.5±2.1	31.3±2.1
20	PS40	92.4±2.6	89.7±2.6	83.3±1.6	96.6±2.4	95.9±3.4	90.4±2.6
21	PS46	23.6±2.5	22.3±3.4	20.7±1.6	37.7±2.1	35.5±2.6	34.7±2.1
22	PS47	94.3±1.0	90.4±2.6	82.0±2.6	94.2±0.5	89.4±3.4	82.5±0.6
23	PS48	60.7±1.5	59.3±3.4	58.1±3.5	44.5±2.6	43.4±2.1	43.2±0.1
24	PS49	48.5±2.3	46.8±3.4	45.4±2.8	42.2±2.4	41.3±1.6	39.8±2.6
25	PS52	41.9±1.8	40.6±2.6	38.3±1.9	33.4±2.6	31.9±1.4	30.7±0.6
26	PS55	29.7±1.4	27.3±3.2	25.7±2.1	20.4±1.6	19.3±2.1	17.8±1.6
27	PS56	42.2±1.9	39.6±0.6	38.3±3.2	37.2±1.2	35.5±2.4	34.9±2.5
28	PS57	97.1±1.8	93.5±5.3	86.7±3.6	94.6±1.6	89.9±2.6	79.2±0.6
29	PS60	95.8±2.6	88.8±2.6	84.5±4.0	95.0±0.6	82.4±2.4	81.6±0.1
30	PS68	54.5±2.4	53.6±0.1	52.4±2.6	50.2±3.4	51.0±1.6	48.2±2.0
31	PS69	98.5±2.6	93.3±1.6	89.0±2.1	88.6±5.4	82.4±0.9	83.3±2.4
32	PS70	94.0±2.9	87.9±0.6	80.8±2.6	94.2±0.6	89.4±0.4	82.5±2.1
33	PS74	48.7±2.5	48.1±0.1	47.0±3.6	43.5±2.9	43.2±0.1	41.4±2.3
34	PS75	36.3±2.6	34.3±1.6	30.0±2.4	24.4±1.2	22.5±0.6	21.0±0.4
35	PS77	98.0±2.4	93.3±3.1	90.2±1.6	90.8±0.6	85.9±2.4	84.4±0.6
36	PS78	61.7±1.5	59.0±5.5	55.5±1.6	46.1±2.4	45.7±2.6	44.8±1.6

37	PS79	53.8±1.6	52.5±2.1	51.9±2.0	36.5±1.3	35.5±2.4	33.5±1.8
38	PS82	21.0±1.8	20.4±0.1	19.4±2.0	28.8±2.6	26.9±3.1	25.8±2.1
39	PS91	96.0±1.5	92.3±0.1	91.4±0.8	95.1±2.3	90.2±5.1	85.9±2.6

Table S3. Preliminary screening showing the *in vitro* probiotic attributes of isolated probiotic strains from fermented barnyard millet

S. No.	Isolates	Auto aggregation (%)	Co-aggregation (%)		Cell surface hydrophobicity (%)
			with *Pa*	with *Sa*	
1	PS4	29.0±2.1	26.3±2.1	28.4±0.6	28.0±1.6
2	PS5	27.6±0.6	38.4±0.6	28.0±0.6	21.9±0.6
3	PS7	32.2±1.6	21.2±3.6	19.6±1.6	26.6±0.4
4	PS8	27.1±2.4	28.9±2.1	26.2±1.2	34.4±2.1
5	PS9	25.6±3.2	19.7±2.1	19.0±1.4	35.4±1.6
6	PS10	56.2±4.4	58.7±2.3	56.7±1.6	64.1±3.6
7	PS16	18.4±2.1	23.7±0.9	25.8±2.4	22.8±1.1
8	PS19	27.9±1.9	38.6±1.5	39.2±2.1	48.8±2.4
9	PS20	27.0±1.4	20.9±1.6	19.9±2.6	30.9±1.6
10	PS21	22.3±1.3	22.3±0.9	20.8±2.4	22.2±0.9
11	PS22	25.2±1.7	24.1±0.8	23.3±1.6	25.6±0.4
12	PS24	33.2±2.1	24.2±1.1	25.5±1.9	35.3±0.4
13	PS25	28.5±0.5	21.3±1.2	25.3±1.2	22.1±0.9
14	PS26	24.5±0.9	26.9±2.3	22.5±1.6	20.5±1.2
15	PS27	21.5±2.1	19.1±1.0	16.0±2.4	25.4±1.6
16	PS28	58.2±0.6	58.9±0.6	55.7±2.1	61.4±3.4
17	PS30	25.2±1.8	23.6±3.6	19.5±2.4	31.8±2.4
18	PS31	36.6±1.5	17.8±1.6	16.1±3.2	24.4±3.4
19	PS32	25.0±0.6	17.8±1.4	16.1±3.1	34.7±2.3
20	PS40	52.4±1.4	57.8±2.3	56.1±2.1	62.8±2.4
21	PS46	22.4±1.6	22.3±2.9	26.8±2.6	25.4±2.1
22	PS47	59.9±0.6	62.9±1.8	58.3±1.4	63.4±1.6
23	PS48	36.8±0.7	23.6±0.9	17.9±2.6	26.6±1.1
24	PS49	42.7±2.1	27.2±2.7	28.7±3.1	32.8±1.0
25	PS52	37.0±2.3	21.9±3.1	37.7±1.4	21.7±1.4
26	PS55	35.6±3.1	22.7±2.7	18.5±0.4	41.6±2.1
27	PS56	20.1±1.6	13.2±0.6	19.9±0.2	28.5±1.6
28	PS57	64.4±1.7	63.6±4.5	59.1±2.1	59.3±3.2
29	PS60	61.0±0.6	53.8±2.6	55.1±2.6	60.0±3.6
30	PS68	22.3±1.8	22.7±1.4	29.6±1.1	25.9±2.1

31	PS69	56.1±2.4	68.3±1.4	60.0±1.6	60.6±0.2
32	PS70	59.9±2.9	63.6±1.9	61.1±2.1	61.6±1.6
33	PS74	27.3±2.4	17.4±1.4	15.3±2.4	28.3±2.4
34	PS75	33.5±3.1	27.1±0.9	21.8±1.6	40.2±2.1
35	PS77	58.2±4.5	67.4±2.4	62.5±2.4	61.4±1.8
36	PS78	32.9±5.4	27.2±2.1	22.1±2.1	20.1±1.9
37	PS79	22.2±0.9	23.9±3.1	19.6±1.6	35.7±2.4
38	PS82	28.1±0.2	28.0±2.6	23.3±3.4	24.4±2.1
39	PS91	62.4±0.6	58.0±2.8	53.3±2.6	62.8±0.6

Table S4. Preliminary screening showing the *in vitro* antioxidant properties of selected isolates

S. No.	Isolates	Antioxidant activity (% inhibition)		
		DPPH	Superoxide	Reducing power
1	PS4	11.9±0.1	14.3±0.3	10.2±0.1
2	PS5	5.6±0.1	11.7±0.4	9.3±0.2
3	PS7	10.7±0.2	10.4±0.2	10.7±0.1
4	PS8	5.7±0.1	5.2±0.1	12.3±0.1
5	PS9	11.0±1.1	12.1±0.4	12.4±1.0
6	PS10	56.0±1.6	56.8±2.6	53.0±2.6
7	PS16	14.5±1.2	7.9±0.1	1.5±2.6
8	PS19	13.1±0.4	12.3±1.1	11.2±2.6
9	PS20	8.7±0.6	12.0±0.6	19.9±1.1
10	PS21	13.0±0.2	8.4±0.6	11.9±0.3
11	PS22	9.8±1.1	10.2±0.6	12.6±0.2
12	PS24	12.5±0.6	12.0±1.0	10.7±0.3
13	PS25	13.1±0.6	17.3±1.2	9.9±0.6
14	PS26	10.3±0.4	8.0±1.0	8.7±0.9
15	PS27	13.8±0.2	7.9±0.2	5.3±0.1
16	PS28	53.1±1.6	53.2±2.1	57.8±2.4
17	PS30	9.6±0.2	12.3±1.6	10.7±0.3
18	PS31	11.6±0.4	11.6±1.6	12.6±0.2
19	PS32	14.4±0.2	3.9±0.1	10.0±1.0
20	PS40	51.3±0.1	58.8±2.4	58.0±1.0
21	PS46	12.4±0.7	12.0±1.0	3.5±0.2
22	PS47	55.7±0.1	56.6±2.6	58.5±2.1
23	PS48	11.1±0.2	7.0±0.1	10.5±0.2
24	PS49	12.6±1.1	11.2±0.1	5.7±0.1
25	PS52	11.7±1.5	10.2±1.1	8.3±0.1
26	PS55	13.6±1.3	7.0±1.0	9.7±0.2
27	PS56	10.8±1.2	9.9±1.1	5.9±0.1
28	PS57	54.6±2.5	55.7±2.4	52.5±2.1
29	PS60	63.9±2.6	63.9±2.3	61.6±0.4

30	PS68	14.6±0.3	8.0±1.0	8.0±0.2
31	PS69	62.5±0.7	53.6±1.0	56.2±0.4
32	PS70	67.4±2.5	54.6±1.6	57.1±2.4
33	PS74	14.0±0.4	11.0±1.0	10.5±0.2
34	PS75	15.4±0.2	12.0±1.0	5.8±0.6
35	PS77	65.7±3.1	51.5±2.1	59.6±2.1
36	PS78	10.8±0.2	8.6±0.6	4.2±0.1
37	PS79	16.9±2.1	9.5±0.4	5.8±0.4
38	PS82	13.5±0.1	18.6±1.1	5.9±0.2
39	PS91	57.4±2.1	58.1±2.4	58.0±2.6

Table S5. Preliminary screening showing the *in vitro* antioxidant properties of selected isolates

S. No.	Isolates	Antioxidant activity (% inhibition)			
		Hydroxide	ABTS	β-Carotene bleaching	Ascorbate autoxidation
1	PS4	10.5±0.2	3.6±1.1	7.1±1.1	6.1±0.2
2	PS5	9.4±0.4	5.1±1.1	10.9±1.2	5.1±0.2
3	PS7	9.8±0.1	11.7±1.0	6.1±0.2	14.2±1.1
4	PS8	3.4±0.1	3.8±1.1	3.3±0.1	8.8±0.2
5	PS9	8.5±0.1	10.6±1.2	10.9±0.1	11.1±1.2
6	PS10	67.7±0.4	61.0±2.4	54.3±2.6	60.8±0.2
7	PS16	9.1±0.3	9.3±1.2	12.3±1.1	11.8±0.2
8	PS19	6.8±0.2	8.4±1.6	4.8±0.2	10.9±0.1
9	PS20	7.5±0.1	12.7±1.2	6.3±0.1	11.6±0.4
10	PS21	6.5±0.3	9.9±1.4	5.8±0.1	7.4±0.2
11	PS22	5.0±0.0	8.6±0.3	15.2±2.1	6.1±1.1
12	PS24	7.5±0.1	8.4±2.1	11.3±1.1	6.6±0.2
13	PS25	4.3±0.3	10.7±1.1	14.4±1.2	13.2±2.1
14	PS26	13.9±0.1	8.6±1.6	6.7±0.2	6.1±0.1
15	PS27	8.7±0.6	7.7±2.1	5.2±0.1	8.2±2.1
16	PS28	50.0±2.1	59.3±3.1	52.3±2.6	57.5±2.4
17	PS30	10.2±0.3	7.0±1.1	6.0±2.1	9.6±1.6
18	PS31	11.6±0.1	12.7±1.2	6.7±0.1	10.2±1.4
19	PS32	10.8±0.2	7.5±0.5	5.0±2.1	12.5±1.1
20	PS40	56.7±2.4	54.9±2.3	63.9±2.3	58.2±2.6
21	PS46	11.4±1.5	12.4±0.4	7.1±0.1	9.9±0.5
22	PS47	52.3±1.2	58.2±1.6	10.9±0.7	60.7±2.4
23	PS48	15.0±0.5	7.5±0.5	11.3±1.2	7.3±0.4
24	PS49	9.7±1.2	7.6±0.6	10.8±1.4	10.1±1.1
25	PS52	13.5±0.6	9.2±1.1	8.5±1.3	6.7±0.4
26	PS55	12.8±2.1	6.1±1.1	7.1±2.1	11.1±0.2
27	PS56	9.5±0.1	8.3±0.2	13.0±2.0	5.8±0.1
28	PS57	55.9±2.3	58.1±1.2	54.7±1.6	58.1±2.1
29	PS60	53.6±1.1	61.9±0.6	65.6±2.4	61.9±2.7

30	PS68	9.6±0.6	9.4±1.3	10.8±1.1	12.1±0.2
31	PS69	58.7±1.1	61.5±1.2	53.0±0.8	62.9±1.6
32	PS70	51.9±0.1	60.3±2.3	40.7±2.4	64.9±2.4
33	PS74	10.2±2.1	11.4±1.6	13.0±1.6	8.9±0.1
34	PS75	7.0±0.2	10.4±1.4	6.4±0.2	12.9±2.1
35	PS77	58.1±0.4	60.7±2.6	54.3±2.4	63.2±2.4
36	PS78	6.8±0.3	5.8±2.4	7.0±0.2	8.3±0.2
37	PS79	13.6±0.2	9.4±2.1	10.2±0.1	14.4±0.2
38	PS82	3.8±0.2	5.9±2.4	15.0±0.6	11.0±0.4
39	PS91	53.7±2.5	54.9±2.1	54.1±1.6	57.4±2.6

Table S6. The initial assessment demonstrated the *in vitro* anti-pathogenic activities of the probiotic strains isolated from fermented barnyard millet

S. No.	Isolates	Inhibition Zone (mm)	
		P. aeruginosa	***S. aureus***
1	PS4	2.0±0.9	2.3±0.7
2	PS5	2.6±0.6	2.3±1.6
3	PS7	0 .0±0.0	0.0±0.0
4	PS8	2.0±1.2	2.0±1.0
5	PS9	2.0±1.3	0.0±0.0
6	PS10	6.3±0.5	6.7±0.5
7	PS16	0.0±0.0	2.0±0.5
8	PS19	3.6±1.5	3.3±1.6
9	PS20	2.0±1.4	2.3±1.0
10	PS21	0.0±0.0	0.0±0.0
11	PS22	3.0±1.1	3.6±1.6
12	PS24	2.3±0.5	2.0±1.8
13	PS25	2.0±0.4	0.0±0.6
14	PS26	0.0±0.0	2.0±0.6
15	PS27	3.0±1.6	3.0±1.0
16	PS28	7.6±1.6	6.3±1.9
17	PS30	3.3±0.6	3.0±1.6
18	PS31	2.0±1.7	0.0±0.0
19	PS32	2.0±0.6	3.0±0.7
20	PS40	7.6±1.6	7.3±0.9
21	PS46	0.0±0.0	0.0±0.0
22	PS47	6.3±0.7	6.6±0.6
23	PS48	0.0±0.0	2.0±1.8
24	PS49	3.0±0.6	2.0±0.6
25	PS52	2.3±1.2	0.0±0.0
26	PS55	3.0±0.6	2.6±0.8
27	PS56	0.0±0.0	2.0±1.0
28	PS57	8.0±1.7	7.0±0.0
29	PS60	6.6±1.5	6.3±0.5
30	PS68	2.0±1.8	3.0±0.0

31	PS69	6.2±0.4	7.2±0.7
32	PS70	8.3±1.3	8.0±0.2
33	PS74	2.6±0.5	3.0±0.6
34	PS75	0.0±0.0	2.0±0.0
35	PS77	6.3±0.6	7.0±0.9
36	PS78	3.0±0.6	2.0±1.0
37	PS79	2.3±1.0	0.0±0.0
38	PS82	0.0±0.0	2.0±0.3
39	PS91	8.3±0.7	7.0±1.2

Table S7. Lifespan analyses – Monocultures

Conditions		MS (days±SEM)	N	Censored	% Change	p Value
E. coli	OP50	20.189±0.422	163	11		
B. licheniformis (BL)	PS70	22.834±0.467	139	5	(+) 13.10	<0.0001
B. licheniformis (BL)	PS74	20.867±0.504	143	0	(+) 3.4	0.0698
B. licheniformis (BL)	PS75	21.175±0.498	154	5	(+) 4.88	0.0572
E. faecium (EF)	PS7	20.216±0.481	141	9	(+) 0.14	0.3164
E. faecium (EF)	PS21	20.052±0.487	150	15	(-) 0.68	0.5002
E. italicus (EI)	PS27	19.070±0.0494	125	2	(-) 5.54	0.3040
E. italicus (EI)	PS56	21.119±0.566	130	2	(+) 4.61	0.0642
L. rhamnosus (LR)	PS25	19.189±0.447	140	8	(-) 4.95	0.1148
L. rhamnosus (LR)	PS26	20.602±0.397	170	14	(+) 2.05	0.8592
L. rhamnosus (LR)	PS28	23.183±0.532	136	7	(+) 14.83	<0.0001
L. rhamnosus (LR)	PS31	20.666±0.507	153	10	(+) 2.36	0.0654
L. rhamnosus (LR)	PS32	21.060±0.382	180	11	(+) 4.31	0.2132
L. plantarum (LP)	PS46	21.237±0.506	127	3	(+) 5.19	0.0385
L. plantarum (LP)	PS57	22.896±0.592	134	12	(+) 13.41	<0.0001
L. amylovorus (LA)	PS49	20.652±0.579	125	8	(+) 2.29	0.0853
L. amylovorus (LA)	PS55	21.877±0.494	146	11	(+) 8.36	0.0005
L. amylovorus (LA)	PS60	23.311±0.661	130	10	(+) 15.46	<0.0001
L. amylovorus (LA)	PS68	21.579±0.491	137	10	(+) 6.87	0.0013
L. delbrueckii subsp. *bulgaricus*	PS69	21.721±0.510	135	5	(+) 12.54	<0.0001
L. delbrueckii subsp. *bulgaricus*	PS77	22.730±0.524	131	4	(+) 12.59	<0.0001
L. delbrueckii subsp. *bulgaricus*	PS78	21.363±0.546	132	4	(+) 5.82	0.0026
L. delbrueckii subsp. *bulgaricus*	PS79	21.136±0.553	140	8	(+) 4.69	0.0086
L. lactis (LL)	PS4	21.024±0.426	156	10	(+) 4.14	0.1499
L. lactis (LL)	PS5	20.289±0.477	140	6	(+) 0.50	0.5494
L. lactis (LL)	PS9	20.239±0.440	150	9	(+) 0.25	0.6914
L. lactis (LL)	PS10	22.984±0.491	142	10	(+) 9.91	<0.0001
L. fermentum (LF)	PS40	25.468±0.481	153	8	(+) 26.15	<0.0001
L. fermentum (LF)	PS52	20.909±0.441	139	2	(+) 3.57	0.3352
P pentosaceus (PP)	PS82	21.667±0.400	164	14	(+) 7.32	0.0623
P. pentosaceus (PP)	PS91	25.026±0.494	154	8	(+) 23.96	<0.0001
W. confusa (WC)	PS8	20.389±0.444	146	10	(+) 0.99	0.8146
W. confusa (WC)	PS47	24.541±0.515	151	12	(+) 21.56	<0.0001
W. confusa (WC)	PS48	21.847±0.412	155	12	(+) 8.21	0.0754

Conditions		MS (days±SEM)	N	Censored	% Change	p Value
W. coagulans (WzC)	PS16	20.209±0.434	169	9	(+) 0.10	0.5267
W. coagulans (WzC)	PS19	20.399±0.497	157	6	(+) 1.04	0.0719
W. coagulans (WzC)	PS20	21.255±0.410	174	10	(+) 5.28	0.1229
W. coagulans (WzC)	PS22	20.117±0.403	166	8	(-) 0.36	0.9636
W. coagulans (WzC)	PS24	20.994±0.431	153	7	(+) 3.99	0.3798
W. coagulans (WzC)	PS30	20.302±0.421	177	14	(+) 0.56	0.8005

Table S8. Lifespan analyses – Pair-wise combinations

Conditions		MS (days±SEM)	N	Censored	% Change	*p* Value
E. coli OP50	Control	19.55±0.402	159	6		
BL PS70+LR PS28	Cocktail 1	23.95±0.514	178	10	(+) 22.94	0.0001
BL PS70+LP PS57	Cocktail 2	23.53±0.483	164	15	(+) 20.70	0.0001
BL PS70+LA PS60	Cocktail 3	21.66±0.533	172	11	(+) 11.15	0.0001
BL PS70+LB PS77	Cocktail 4	23.16±0.495	178	8	(+) 18.85	0.0001
BL PS70+LL PS10	Cocktail 5	22.16±0.525	154	5	(+) 13.72	0.0001
BL PS70+LF PS40	Cocktail 6	23.09±0.539	168	16	(+) 18.44	0.0001
BL PS70+PP PS91	Cocktail 7	22.86±0.572	141	6	(+) 17.31	0.0001
BL PS70+WC PS47	Cocktail 8	22.28±0.528	157	11	(+) 16.90	0.0001
E. coli OP50		19.63±0.402	158	10		
LR PS28+LP PS57	Cocktail 9	22.48±0.486	148	13	(+) 14.52	0.0001
LR PS28+LA PS60	Cocktail 10	22.57±0.460	174	8	(+) 14.93	0.0001
LR PS28+LB PS77	Cocktail 11	21.67±0.504	157	15	(+) 10.39	0.0001
LR PS28+LL PS10	Cocktail 12	21.16±0.434	164	11	(+) 7.79	0.0001
LR PS28+LF PS40	Cocktail 13	22.10±0.420	173	13	(+) 12.60	0.0001
LR PS28+PP PS91	Cocktail 14	21.74±0.467	153	10	(+) 10.75	0.0001
LR PS28+WC PS47	Cocktail 15	22.20±0.520	148	8	(+) 13.09	0.0001
E. coli OP50		19.70±0.441	151	12		
LP PS57+LA PS60	Cocktail 16	24.683±0.562	148	7	(+) 29.39	0.0001
LP PS57+LB PS77	Cocktail 17	22.35±0.446	166	10	(+) 13.48	0.0001
LP PS57+LL PS10	Cocktail 18	22.75±0.416	149	12	(+) 15.51	0.0001
LP PS57+LF PS40	Cocktail 19	22.70±0.426	158	8	(+) 15.26	0.0001
LP PS57+PP PS91	Cocktail 20	22.60±0.419	175	20	(+) 14.75	0.0001
LP PS57+WC PS47	Cocktail 21	21.72±0.410	150	14	(+) 10.28	0.0014
E. coli OP50		19.29±0.345	160	11		
LA PS60+LB PS77	Cocktail 22	22.34±0.381	153	14	(+) 15.81	0.0001
LA PS60+LL PS10	Cocktail 23	21.76±0.491	161	12	(+) 12.80	0.0001
LA PS60+LF PS40	Cocktail 24	22.20±0.440	173	15	(+) 15.09	0.0001
LA PS60+PP PS91	Cocktail 25	22.22±0.406	158	10	(+) 15.19	0.0001
LA PS60+WC PS47	Cocktail 26	22.00±0.378	163	11	(+) 14.05	0.0001
E. coli OP50		19.29±0.345	160	11		
LB PS77+LL PS10	Cocktail 27	23.670±0.511	158	10	(+) 22.71	0.0001

Conditions		MS (days±SEM)	N	Censored	% Change	*p* Value
LB PS77+LF PS40	Cocktail 28	21.81±0.415	166	9	(+) 13.06	0.0001
LB PS77+PP PS91	Cocktail 29	21.75±0.365	164	12	(+) 12.75	0.0001
LB PS77+WC PS47	Cocktail 30	21.67±0.386	159	8	(+) 12.34	0.0001
E. coli OP50		19.29±0.345	160	11		
LL PS10+LF PS40	Cocktail 31	21.45±0.375	152	8	(+) 11.20	0.0001
LL PS10+PP PS91	Cocktail 32	21.07±0.381	175	12	(+) 9.23	0.0001
LL PS10+WC PS47	Cocktail 33	20.98±0.400	170	7	(+) 8.76	0.0001
E. coli OP50		19.29±0.345	160	11		
LF PS40+PP PS91	Cocktail 34	21.39±0.386	147	9	(+) 10.89	0.0001
LF PS40+WC PS47	Cocktail 35	22.31±0.430	158	11	(+) 15.66	0.0001
E. coli OP50		19.29±0.345	160	11		
PP PS91+WC PS47	Cocktail 36	22.90±0.417	165	13	(+) 18.71	0.0001

Table S9. Lifespan analyses – Three-way combinations

Conditions		MS (days±SEM)	N	Censored	% Change	*p* Value
E. coli OP50		19.63±0.405	163	6		
PS70+PS28+PS57	Cocktail 37	21.71±0.451	169	7	(+) 10.60	0.0001
PS70+PS28+PS60	Cocktail 38	21.35±0.427	158	10	(+) 8.76	0.0005
PS70+PS28+PS77	Cocktail 39	22.39±0.434	165	12	(+) 14.06	0.0001
PS70+PS28+PS10	Cocktail 40	22.50±0.412	172	10	(+) 14.62	0.0001
PS70+PS28+PS40	Cocktail 41	21.90±0.420	151	9	(+) 11.56	0.0001
PS70+PS28+PS91	Cocktail 42	22.92±0.402	158	12	(+) 16.84	0.0001
PS70+PS28+PS47	Cocktail 43	23.12±0.394	156	14	(+) 17.78	0.0001
PS70+PS57+PS60	Cocktail 44	21.77±0.396	173	9	(+) 10.90	0.0001
PS70+PS57+PS77	Cocktail 45	22.04±0.390	158	10	(+) 12.28	0.0001
PS70+PS57+PS10	Cocktail 46	22.86±0.372	155	7	(+) 16.45	0.0001
PS70+PS57+PS40	Cocktail 47	23.23±0.395	162	11	(+) 18.34	0.0001
PS70+PS57+PS91	Cocktail 48	22.11±0.386	160	9	(+) 12.79	0.0001
PS70+PS57+PS47	Cocktail 49	22.21±0.381	153	7	(+) 12.58	0.0001
PS70+PS60+PS77	Cocktail 50	22.79±0.405	148	6	(+) 16.10	0.0001
PS70+PS60+PS10	Cocktail 51	21.99±0.388	162	7	(+) 12.02	0.0002
PS70+PS60+PS40	Cocktail 52	22.98±0.428	167	11	(+) 17.01	0.0001
PS70+PS60+PS91	Cocktail 53	22.80±0.407	169	6	(+) 16.15	0.0001
PS70+PS60+PS47	Cocktail 54	22.91±0.430	154	5	(+) 16.71	0.0001
PS70+PS77+PS60 (Embryo)	Cocktail 55	28.693±0.507	171	10	(+) 46.8	0.0001
PS70+PS77+PS60 (Young adult)	Cocktail 55	24.771±0.517	170	8	(+) 26.2	0.0001
PS70+PS77+PS60 (Embryo-Young adult)	Cocktail 55	21.068±0.368	156	7	(+) 7.3	0.0627
PS70+PS77+PS40	Cocktail 56	23.25±0.453	175	16	(+) 18.44	0.0001
PS70+PS77+PS91	Cocktail 57	21.84±0.400	180	14	(+) 11.26	0.0001
PS70+PS77+PS47	Cocktail 58	22.01±0.388	160	9	(+) 12.12	0.0001
PS70+PS10+PS40	Cocktail 59	22.56±0.425	161	10	(+) 14.88	0.0001

Conditions		MS (days±SEM)	N	Censored	% Change	*p* Value
PS70+PS10+PS91	Cocktail 60	22.77±0.395	167	12	(+) 16.00	0.0001
PS70+PS10+PS47	Cocktail 61	21.79±0.419	156	10	(+) 11.00	0.0001
PS70+PS40+PS91	Cocktail 62	22.60±0.488	153	9	(+) 15.13	0.0001
PS70+PS40+PS47	Cocktail 63	22.14±0.452	171	17	(+) 12.79	0.0001
PS70+PS91+PS47	Cocktail 64	22.76±0.466	160	15	(+) 15.94	0.0001
E. coli OP50		19.58±0.394	164	6		
PS28+PS57+PS60	Cocktail 65	22.44±0.416	174	9	(+) 14.2	0.0001
PS28+PS57+PS77	Cocktail 66	23.46±0.465	153	12	(+) 19.93	0.0001
PS28+PS57+PS10	Cocktail 67	22.62±0.431	162	16	(+) 15.64	0.0001
PS28+PS57+PS40	Cocktail 68	22.24±0.444	164	19	(+) 13.70	0.0001
PS28+PS57+PS91	Cocktail 69	22.82±0.458	168	17	(+) 16.67	0.0001
PS28+PS57+PS47	Cocktail 70	22.79±0.453	171	14	(+) 16.51	0.0001
PS28+PS60+PS77	Cocktail 71	22.53±0.454	169	16	(+) 15.18	0.0001
PS28+PS60+PS10	Cocktail 72	23.21±0.408	158	14	(+) 18.66	0.0001
PS28+PS60+PS40	Cocktail 73	23.09±0.427	167	11	(+) 18.07	0.0001
PS28+PS60+PS91	Cocktail 74	22.26±0.440	156	9	(+) 13.81	0.0001
PS28+PS60+PS47	Cocktail 75	22.81±0.422	166	11	(+) 16.62	0.0001
PS28+PS77+PS10	Cocktail 76	22.90±0.455	159	13	(+) 17.02	0.0001
PS28+PS77+PS40	Cocktail 77	23.52±0.452	165	10	(+) 18.87	0.0001
PS28+PS77+PS91	Cocktail 78	23.14±0.465	157	11	(+) 18.30	0.0001
PS28+PS77+PS47	Cocktail 79	22.88±0.497	151	14	(+) 16.97	0.0001
PS28+PS10+PS40	Cocktail 80	23.20±0.469	162	13	(+) 18.61	0.0001
PS28+PS10+PS91	Cocktail 81	22.55±0.467	155	10	(+) 15.29	0.0001
PS28+PS10+PS47	Cocktail 82	23.28±0.466	151	12	(+) 19.02	0.0001
PS28+PS40+PS91	Cocktail 83	22.07±0.459	157	14	(+) 12.83	0.0001
PS28+PS40+PS47	Cocktail 84	22.72±0.462	153	12	(+) 16.16	0.0001
PS28+PS91+PS47	Cocktail 85	23.07±0.454	162	17	(+) 17.94	0.0001
E. coli OP50		19.74±0.391	168	7		
PS57+PS60+PS77	Cocktail 86	23.17±0.435	161	11	(+) 17.22	0.0001
PS57+PS60+PS10	Cocktail 87	22.68±0.462	160	13	(+) 14.89	0.0001
PS57+PS60+PS40	Cocktail 88	22.10±0.475	156	12	(+) 11.96	0.0001
PS57+PS60+PS91	Cocktail 89	22.61±0.473	154	13	(+) 14.54	0.0001
PS57+PS60+PS47	Cocktail 90	22.40±0.499	164	10	(+) 13.48	0.0001
PS57+PS77+PS10	Cocktail 91	22.87±0.467	152	7	(+) 15.86	0.0001
PS57+PS77+PS40	Cocktail 92	23.53±0.491	158	9	(+) 19.20	0.0001
PS57+PS77+PS91	Cocktail 93	22.59±0.501	169	11	(+) 14.44	0.0001
PS57+PS77+PS47	Cocktail 94	22.79±0.498	162	10	(+) 15.45	0.0001
PS57+PS10+PS40	Cocktail 95	23.29±0.482	145	7	(+) 17.98	0.0001
PS57+PS10+PS91	Cocktail 96	23.41±0.508	147	10	(+) 18.59	0.0001
PS57+PS10+PS47	Cocktail 97	22.07±0.440	155	9	(+) 11.80	0.0001
PS57+PS40+PS91	Cocktail 98	22.88±0.480	157	13	(+) 15.59	0.0001
PS57+PS40+PS47	Cocktail 99	23.37±0.371	145	9	(+) 18.39	0.0001
PS57+PS91+PS47	Cocktail 100	21.17±0.447	163	15	(+) 7.24	0.0036
E. coli OP50		19.39±0.414	162	6		
PS60+PS77+PS10	Cocktail 101	21.08±0.414	168	12	(+) 8.75	0.0008
PS60+PS77+PS40	Cocktail 102	21.93±0.366	163	10	(+) 13.10	0.0001
PS60+PS77+PS91	Cocktail 103	22.65±0.420	172	23	(+) 16.81	0.0001
PS60+PS77+PS47	Cocktail 104	22.57±0.489	168	8	(+) 16.43	0.0001

Conditions		MS (days±SEM)	N	Censored	% Change	*p* Value
PS60+PS10+PS40	Cocktail 105	22.40±0.529	158	15	(+) 15.52	0.0001
PS60+PS10+PS91	Cocktail 106	22.46±0.565	136	6	(+) 15.83	0.0001
PS60+PS10+PS47	Cocktail 107	22.29±0.484	145	12	(+) 14.96	0.0001
PS60+PS40+PS91	Cocktail 108	21.53±0.502	155	14	(+) 11.04	0.0001
PS60+PS40+PS47	Cocktail 109	22.20±0.520	148	10	(+) 14.49	0.0001
PS60+PS91+PS47	Cocktail 110	22.87±0.430	161	13	(+) 17.95	0.0001
E. coli OP50		19.42±0.353	162	10		
PS77+PS10+PS40	Cocktail 111	22.48±0.433	161	12	(+) 15.62	0.0001
PS77+PS10+PS91 (Embryo)	Cocktail 112	29.74±0.531	175	15	(+) 53.1	0.0001
PS77+PS10+PS91 (Young adult)	Cocktail 112	25.91±0.539	150	2	(+) 33.4	0.0001
PS77+PS10+PS91 (Embryo-Young adult)	Cocktail 112	20.73±0.422	162	10	(+) 6.7	0.0016
PS77+PS10+PS47	Cocktail 113	21.73±0.403	152	9	(+) 12.20	0.0001
PS77+PS40+PS91	Cocktail 114	22.05±0.423	170	13	(+) 13.54	0.0001
PS77+PS40+PS47	Cocktail 115	21.75±0.365	164	8	(+) 12.00	0.0001
PS77+PS91+PS47	Cocktail 116	22.64±0.384	181	14	(+) 16.58	0.0001
E. coli OP50		19.42±0.353	162	10		
PS10+PS40+PS91	Cocktail 117	22.04±0.427	151	10	(+) 13.49	0.0001
PS10+PS40+PS47	Cocktail 118	23.02±0.377	158	8	(+) 18.54	0.0001
PS10+PS91+PS47	Cocktail 119	22.05±0.384	159	9	(+) 13.54	0.0001
PS40+PS91+PS47	Cocktail 120	22.78±0.474	157	14	(+) 17.30	0.0001

Table S10. Lifespan analyses – genetic mutants

Genotype	Conditions	MS (days±SEM)	N	Censored	% Change	*p* Value
daf-2(*e1370*)	Control	31.777±0.845	123	10		
	Cocktail 55	44.587±0.801	134	14	(+) 40.31	0.0001
	Cocktail 112	31.652±0.996	113	11	(-) 0.39	0.6154
age-1(*hx546*)	Control	24.280±0.648	140	14		
	Cocktail 55	33.899±0.725	125	7	(+) 39.62	0.0001
	Cocktail 112	26.792±0.746	134	8	(+) 6.23	0.0726
daf-16(*mu86*)	Control	13.751±0.355	97	5		
	Cocktail 55	19.461±0.565	100	12	(+) 41.52	0.0001
	Cocktail 112	14.409±0.374	106	9	(+) 4.79	0.0359
skn-1(*zu67*)	Control	13.111±0.273	109	13		
	Cocktail 55	13.161±0.322	102	8	(+) 0.38	0.4681
	Cocktail 112	13.489±0.318	114	11	(+) 2.88	0.1029
hsf-1(*sy441*)	Control	12.945±0.335	99	2		
	Cocktail 55	17.532±0.450	100	5	(+) 37.75	0.0001
	Cocktail 112	17.387±0.484	97	2	(+) 42.04	0.0001
jnk-1(*gk7*)	Control	14.899±0.291	130	10		
	Cocktail 55	21.309±0.346	132	15	(+) 43.02	0.0001
	Cocktail 112	22.288±0.424	120	8	(+) 49.59	0.0001
mpk-1(*ku1*)	Control	15.084±0.341	112	5		
	Cocktail 55	20.214±0.508	122	10	(+) 37.32	0.0001
	Cocktail 112	20.295±0.535	115	4	(+) 41.18	0.0001
pmk-1(*km25*)	Control	15.922±0.315	130	7		
	Cocktail 55	16.072±0.373	122	10	(+) 0.94	0.1288
	Cocktail 112	18.230±0.357	140	7	(+) 14.50	0.0001
tir-1(*ok1052*)	Control	17.593±0.406	117	6		
	Cocktail 55	17.475±0.434	126	5	(-) 0.67	0.5654
	Cocktail 112	20.585±0.399	126	10	(+) 17.01	0.0001
nsy-1(*ag3*)	Control	15.398±0.325	132	8		
	Cocktail 55	15.337±0.323	125	7	(-) 0.40	0.7614
	Cocktail 112	18.478±0.283	113	10	(+) 20.00	0.0001
sek-1(*km4*)	Control	15.890±0.263	116	6		
	Cocktail 55	16.109±0.318	130	11	(+) 1.38	0.0525
	Cocktail 112	19.277±0.349	110	4	(+) 21.32	0.0001
sir-2.1(*ok434*)	Control	16.056±0.345	120	12		
	Cocktail 55	20.972±0.581	112	8	(+) 30.62	0.0001
	Cocktail 112	23.107±0.552	125	12	(+) 43.92	0.0001
aak-2(*ok524*)	Control	16.234±0.349	118	6		
	Cocktail 55	21.063±0.445	110	7	(+) 29.75	0.0001
	Cocktail 112	21.229±0.452	106	6	(+) 30.77	0.0001
eat-2(*ad1116*)	Control	21.324±0.501	130	12		
	Cocktail 55	29.361±0.540	134	10	(+) 37.69	0.0001
	Cocktail 112	28.180±0.517	122	10	(+) 32.15	0.0001
bar-1(*ga80*)	Control	12.779±0.384	108	8		
	Cocktail 55	18.110±0.430	112	8	(+) 41.72	0.0001
	Cocktail 112	15.851±0.350	126	5	(+) 24.04	0.0001
dbl-1(*nk3*)	Control	12.093±0.338	100	5		

Genotype	Conditions	MS (days±SEM)	N	Censored	% Change	*p* Value
	Cocktail 55	16.583±0.329	105	5	(+) 37.13	0.0001
	Cocktail 112	13.894±0.371	100	7	(+) 14.89	0.0001

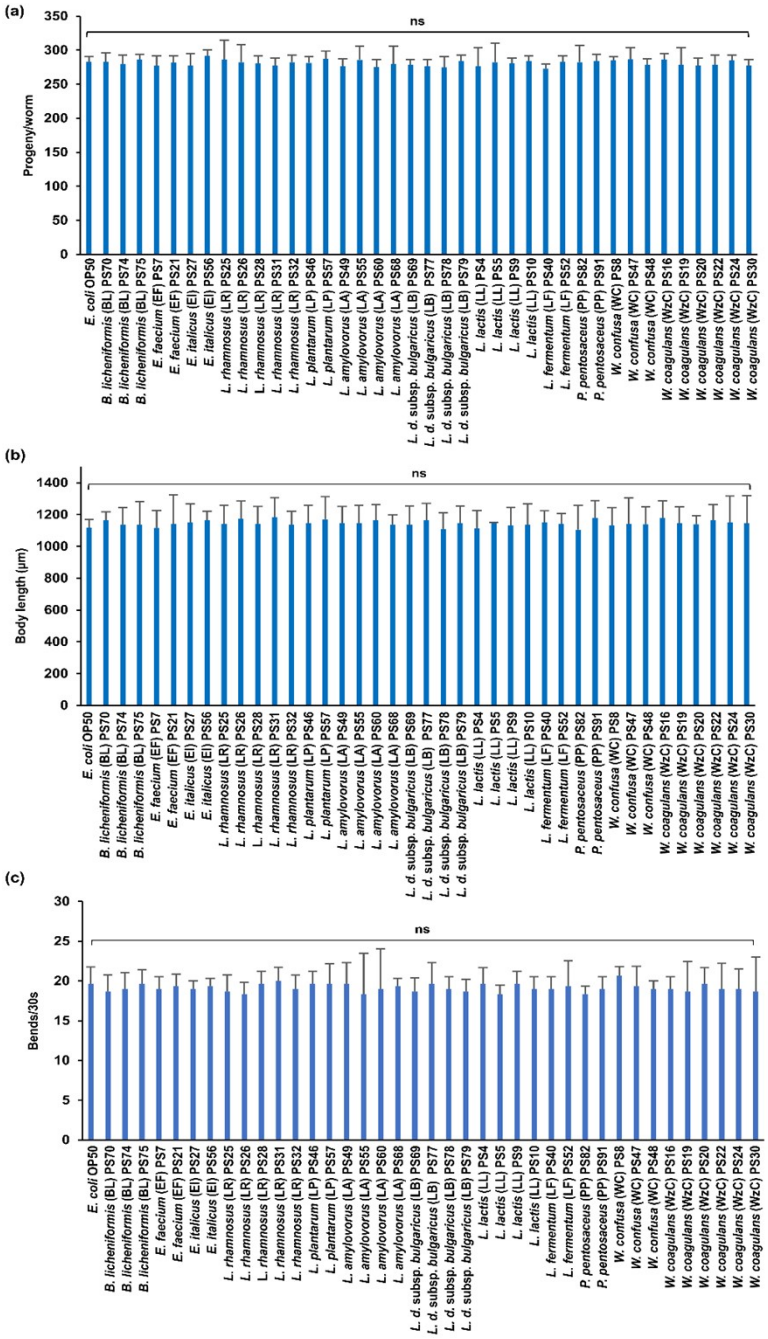
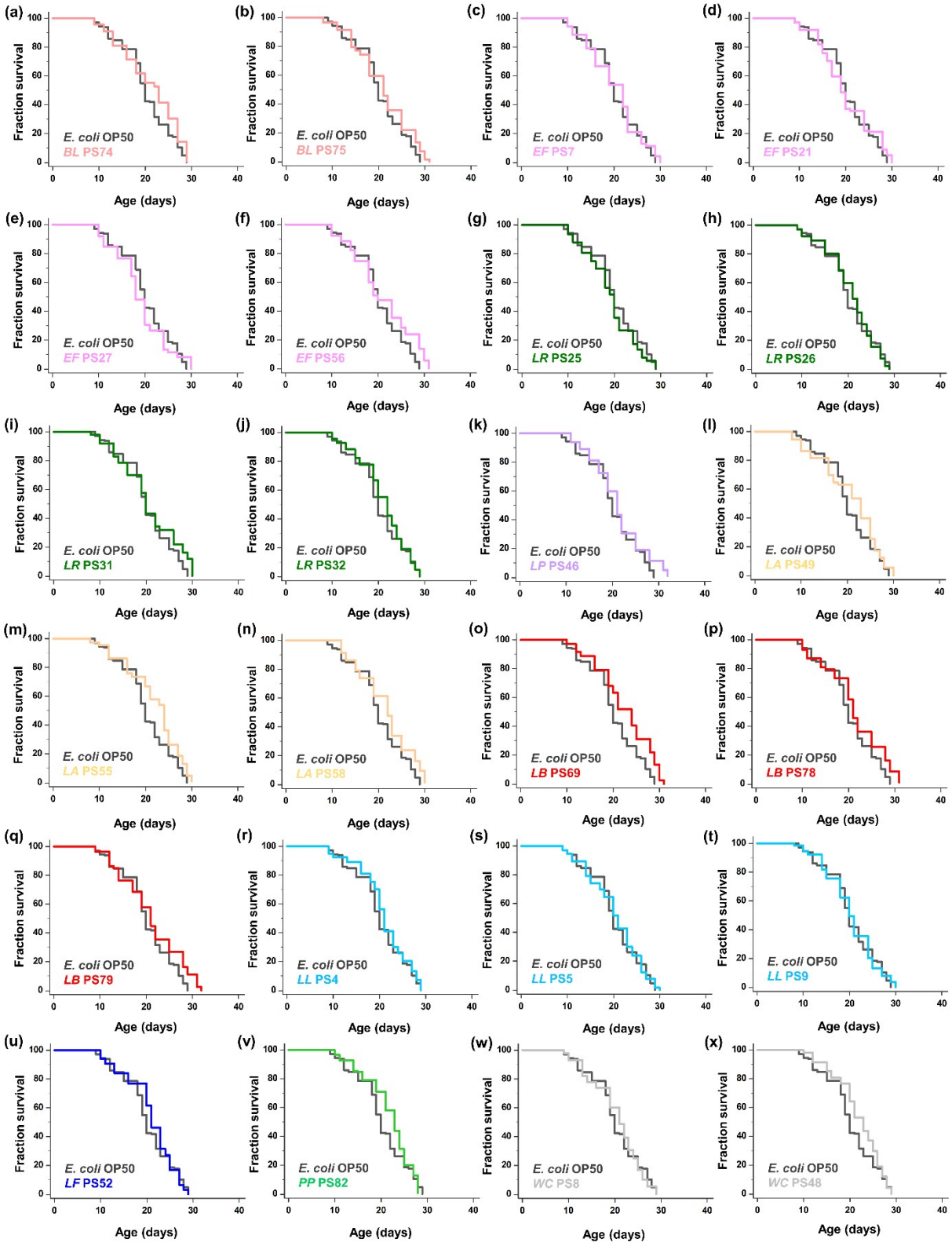


Fig S1. Effect of probiotic supplementation on the (a) reproduction, (b) development, and (c) body bends of *C. elegans*. Bars represent the mean ± SEM of three independent biological trials, each conducted with four replicates under similar conditions as described. ns – not significant vs. *E. coli* OP50.


(a)
E. coli OP50
BL PS74
(b)
E. coli OP50
BL PS75
(c)
E. coli OP50
EF PS7
(d)
E. coli OP50
EF PS21
(e)
E. coli OP50
EF PS27
(f)
E. coli OP50
EF PS56
(g)
E. coli OP50
LR PS25
(h)
E. coli OP50
LR PS26
(i)
E. coli OP50
LR PS31
(j)
E. coli OP50
LR PS32
(k)
E. coli OP50
LP PS46
(l)
E. coli OP50
LA PS49
(m)
E. coli OP50
LA PS55
(n)
E. coli OP50
LA PS58
(o)
E. coli OP50
LB PS69
(p)
E. coli OP50
LB PS78
(q)
E. coli OP50
LB PS79
(r)
E. coli OP50
LL PS4
(s)
E. coli OP50
LL PS5
(t)
E. coli OP50
LL PS9
(u)
E. coli OP50
LF PS52
(v)
E. coli OP50
PP PS82
(w)
E. coli OP50
WC PS8
(x)
E. coli OP50
WC PS48
Fraction survival
Age (days)

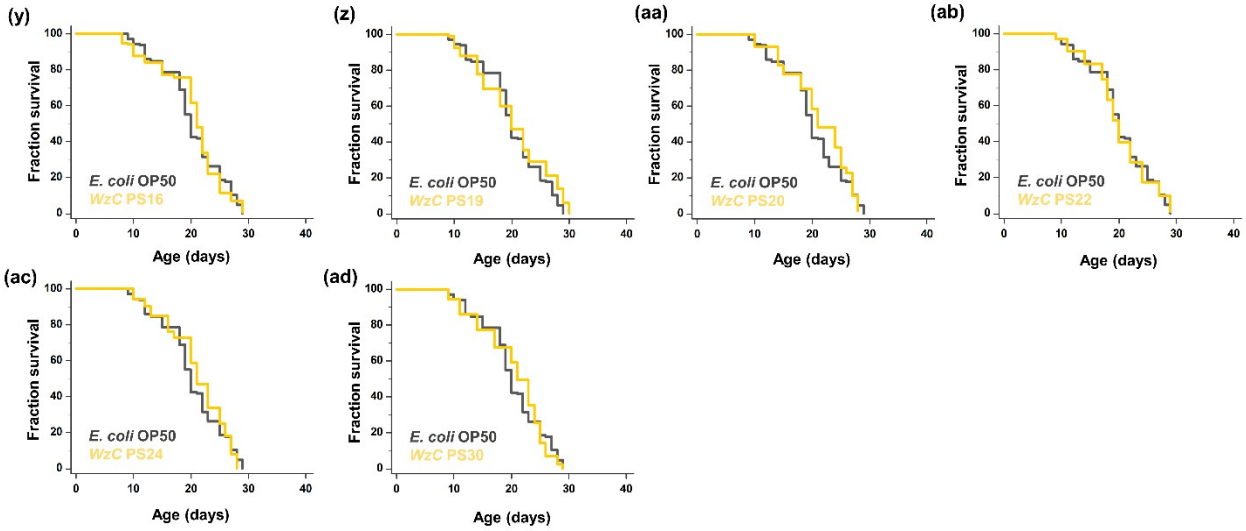


Fig S2. Lifespan analyses. (a-ad) Survivorship curves of wild-type *C. elegans* fed with single strain LAB isolated from traditionally fermented barnyard millet. Combined data from three independent biological trials, performed under similar conditions were presented. Data were analyzed by the Mantel-Cox log-rank test. See Supplementary **Table S8** for statistical details of lifespan analyses.

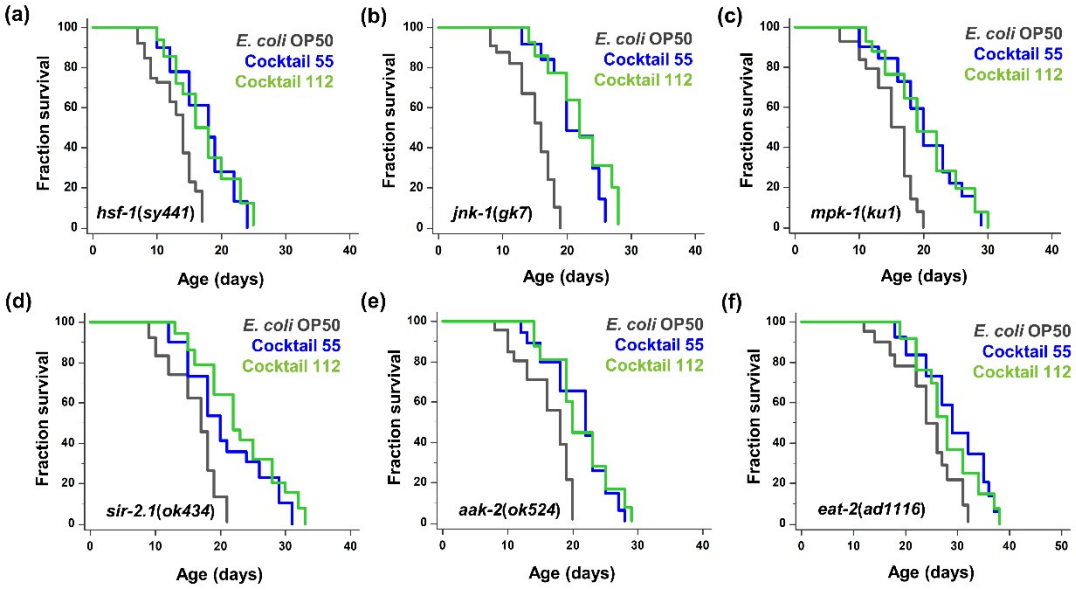


Fig S3. Lifespan analyses. Survivorship curves of (a) *hsf-1*, (b) *jnk-1*, (c) *mpk-1*, (d) *sir-2.1*, (e) *aak-2*, and (f) *eat-2* mutant *C. elegans* fed with gerobiotic cocktails. Combined data from three independent biological trials, performed under similar conditions, were presented. Data were analyzed by the Mantel-Cox log-rank test. See Supplementary **Table S11** for statistical details of lifespan analyses.

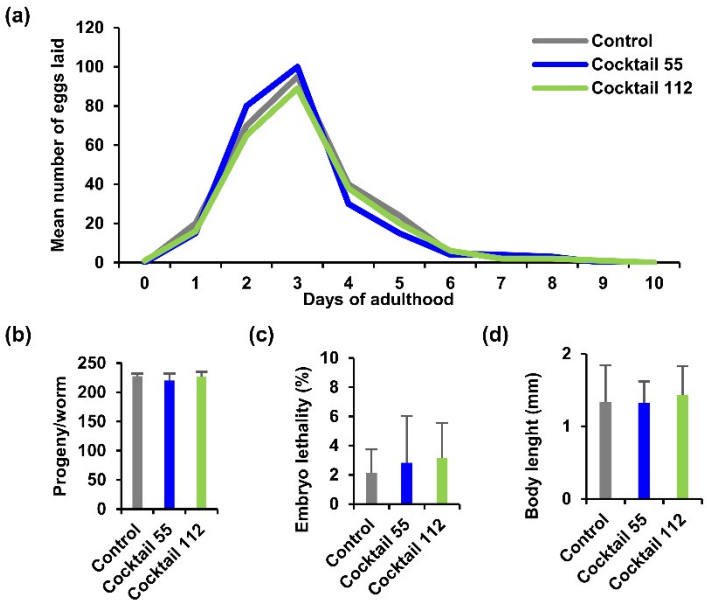


Fig S4. Effect of gerobiotic cocktails on the (a) mean egg production, (b) brood size, (c) embryonic lethality, and (d) development of wild-type *C. elegans*. Gerobiotic cocktail feeding did not adversely affect any of the physiological functions.

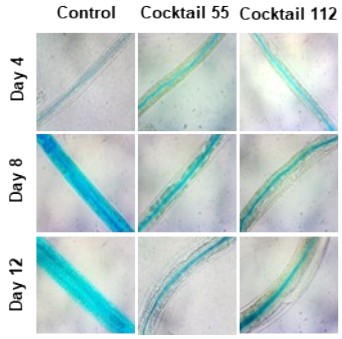


Fig S5. DIC images of wild-type worms after being stained with blue food dye under 40 × magnification. This figure is associated with the main text, Fig. 5a.