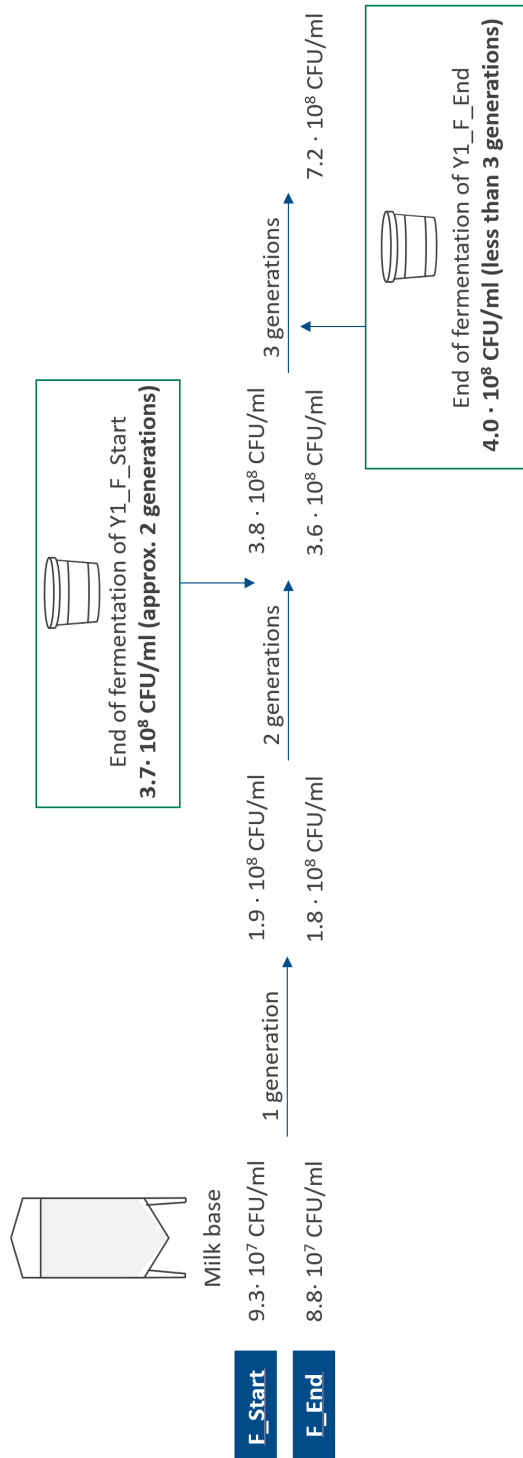


Supplementary figure 1. Survival of *L. rhamnosus* GG in yogurt when incubated in 1% porcine bile for 1 hour. Samples included frozen *L. rhamnosus* GG added to yogurt immediately before treatment (Frozen Lr GG added to Yogurt) and yogurt co-fermented with *L. rhamnosus* GG, both from the day of production (Yogurt with Lr GG – Day 1) and at the end of shelf life (Yogurt with Lr GG – Day 28). Results are shown as CFU/ml (Y-axis) after 7.5, 15, 30 and 60 minutes (X-axis) in 1% porcine bile solution. For each treatment a control is included (X=0).



Supplementary figure 2 Number of generations of *L. rhamnosus* GG when co-fermented in yogurt.

Supplementary table 1 Accession numbers of sequenced data. All DNA sequence data are stored at the National Center for Biotechnology information database under series accession number PRJNA762716.

accession	bioproject_accession	biosample_accession	library_ID	library_layout	instrument_model
SRR15862625	PRJNA762716	SAMN21404543	F_start_ON	paired	Illumina NovaSeq 6000
SRR15862624	PRJNA762716	SAMN21404544	F_start_isolate1	paired	Illumina NovaSeq 6000
SRR15862613	PRJNA762716	SAMN21404545	F_start_isolate10	paired	Illumina NovaSeq 6000
SRR15862602	PRJNA762716	SAMN21404546	F_start_isolate2	paired	Illumina NovaSeq 6000
SRR15862591	PRJNA762716	SAMN21404547	F_start_isolate3	paired	Illumina NovaSeq 6000
SRR15862646	PRJNA762716	SAMN21404548	F_start_isolate4	paired	Illumina NovaSeq 6000
SRR15862635	PRJNA762716	SAMN21404549	F_start_isolate5	paired	Illumina NovaSeq 6000
SRR15862628	PRJNA762716	SAMN21404550	F_start_isolate6	paired	Illumina NovaSeq 6000
SRR15862627	PRJNA762716	SAMN21404551	F_start_isolate7	paired	Illumina NovaSeq 6000
SRR15862626	PRJNA762716	SAMN21404552	F_start_isolate8	paired	Illumina NovaSeq 6000
SRR15862623	PRJNA762716	SAMN21404553	F_start_isolate9	paired	Illumina NovaSeq 6000
SRR15862622	PRJNA762716	SAMN21404554	F_end_ON	paired	Illumina NovaSeq 6000
SRR15862621	PRJNA762716	SAMN21404555	F_end_isolate1	paired	Illumina NovaSeq 6000
SRR15862620	PRJNA762716	SAMN21404556	F_end_isolate10	paired	Illumina NovaSeq 6000
SRR15862619	PRJNA762716	SAMN21404557	F_end_isolate2	paired	Illumina NovaSeq 6000
SRR15862618	PRJNA762716	SAMN21404558	F_end_isolate3	paired	Illumina NovaSeq 6000
SRR15862617	PRJNA762716	SAMN21404559	F_end_isolate4	paired	Illumina NovaSeq 6000
SRR15862616	PRJNA762716	SAMN21404560	F_end_isolate5	paired	Illumina NovaSeq 6000
SRR15862615	PRJNA762716	SAMN21404561	F_end_isolate6	paired	Illumina NovaSeq 6000
SRR15862614	PRJNA762716	SAMN21404562	F_end_isolate7	paired	Illumina NovaSeq 6000
SRR15862612	PRJNA762716	SAMN21404563	F_end_isolate8	paired	Illumina NovaSeq 6000
SRR15862611	PRJNA762716	SAMN21404564	F_end_isolate9	paired	Illumina NovaSeq 6000
SRR15862610	PRJNA762716	SAMN21404565	Y1_F_start_Isolate1	paired	Illumina NovaSeq 6000
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SRR15862608	PRJNA762716	SAMN21404567	Y1_F_start_Isolate2	paired	Illumina NovaSeq 6000
SRR15862607	PRJNA762716	SAMN21404568	Y1_F_start_Isolate3	paired	Illumina NovaSeq 6000
SRR15862606	PRJNA762716	SAMN21404569	Y1_F_start_Isolate4	paired	Illumina NovaSeq 6000
SRR15862605	PRJNA762716	SAMN21404570	Y1_F_start_Isolate5	paired	Illumina NovaSeq 6000
SRR15862604	PRJNA762716	SAMN21404571	Y1_F_start_Isolate6	paired	Illumina NovaSeq 6000
SRR15862603	PRJNA762716	SAMN21404572	Y1_F_start_Isolate7	paired	Illumina NovaSeq 6000
SRR15862601	PRJNA762716	SAMN21404573	Y1_F_start_Isolate8	paired	Illumina NovaSeq 6000
SRR15862600	PRJNA762716	SAMN21404574	Y1_F_start_Isolate9	paired	Illumina NovaSeq 6000
SRR15862599	PRJNA762716	SAMN21404575	Y1_F_start_IsolateON	paired	Illumina NovaSeq 6000
SRR15862598	PRJNA762716	SAMN21404576	Y28_F_start_Isolate1	paired	Illumina NovaSeq 6000
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SRR15862595	PRJNA762716	SAMN21404579	Y28_F_start_Isolate3	paired	Illumina NovaSeq 6000
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SRR15862654	PRJNA762716	SAMN21404585	Y28_F_start_Isolate9	paired	Illumina NovaSeq 6000
SRR15862653	PRJNA762716	SAMN21404586	Y28_F_start_IsolateON	paired	Illumina NovaSeq 6000
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SRR15862640	PRJNA762716	SAMN21404598	Y28_F_end_Isolate1	paired	Illumina NovaSeq 6000
SRR15862639	PRJNA762716	SAMN21404599	Y28_F_end_Isolate10	paired	Illumina NovaSeq 6000
SRR15862638	PRJNA762716	SAMN21404600	Y28_F_end_Isolate2	paired	Illumina NovaSeq 6000
SRR15862637	PRJNA762716	SAMN21404601	Y28_F_end_Isolate3	paired	Illumina NovaSeq 6000
SRR15862636	PRJNA762716	SAMN21404602	Y28_F_end_Isolate4	paired	Illumina NovaSeq 6000
SRR15862634	PRJNA762716	SAMN21404603	Y28_F_end_Isolate5	paired	Illumina NovaSeq 6000
SRR15862633	PRJNA762716	SAMN21404604	Y28_F_end_Isolate6	paired	Illumina NovaSeq 6000
SRR15862632	PRJNA762716	SAMN21404605	Y28_F_end_Isolate7	paired	Illumina NovaSeq 6000
SRR15862631	PRJNA762716	SAMN21404606	Y28_F_end_Isolate8	paired	Illumina NovaSeq 6000
SRR15862630	PRJNA762716	SAMN21404607	Y28_F_end_Isolate9	paired	Illumina NovaSeq 6000
SRR15862629	PRJNA762716	SAMN21404608	Y28_F_end_IsolateON	paired	Illumina NovaSeq 6000

Supplementary table 2 Adjusted p-values from multiple comparison of IL-10, IL-8, IL-6, TNF- α , IL-12p70 and IL-1 β using two-way ANOVA followed by Tukey's test. Cells containing p-values are presented with a color gradient for level of significance as depicted in the key.

	IL-10			IL-8			IL-6			TNF- α			IL-12			IL-1 β			
	Donor A	Donor B	Donor C	Donor A	Donor B	Donor C	Donor A	Donor B	Donor C	Donor A	Donor B	Donor C	Donor A	Donor B	Donor C	Donor A	Donor B	Donor C	
Frozen Lr GG vs. Frozen Lb + St	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0165	<0.0001	<0.0001	0.9808	0.0003	<0.0001	<0.0001	<0.0001	
Frozen Lr GG vs. Frozen Lb + St + Lr GG	0.2253	<0.0001	<0.0001	<0.0001	0.0263	<0.0001	<0.0001	<0.0001	<0.0001	0.0223	0.0182	0.0011	<0.0001	0.9359	<0.0001	0.0002	<0.0001	<0.0001	
Frozen Lr GG vs. Yogurt Lb + St	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9947	0.0326	<0.0001	>0.9999	0.9694	<0.0001	0.0001	<0.0001	
Frozen Lr GG vs. Yogurt Lb + St + Lr GG	<0.0001	0.073	0.9935	<0.0001	0.0386	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	0.866	<0.0001	>0.9999	0.8843	<0.0001	<0.0001	<0.0001	<0.0001	
Frozen Lr GG vs. Negative control	0.9965	0.0577	0.4284	0.0024	0.0054	0.1148	0.854	0.0015	0.0011	0.9502	0.9999	0.9999	>0.9999	>0.9999	0.9999	0.9892	0.7175	0.9995	
Frozen Lb + St vs. Frozen Lb + St + Lr GG	<0.0001	<0.0001	<0.0001	<0.0001	0.0052	0.9978	<0.0001	0.0009	0.8756	<0.0001	>0.9999	0.1531	<0.0001	>0.9999	0.9296	<0.0001	0.8342	<0.0001	
Frozen Lb + St vs. Yogurt Lb + St	0.0952	<0.0001	<0.0001	>0.9999	<0.0001	<0.0001	<0.0001	0.6843	0.4016	0.7045	0.0599	0.0075	<0.0001	0.995	0.0032	0.9133	0.0636	<0.0001	
Frozen Lb + St vs. Yogurt Lb + St + Lr GG	0.0004	<0.0001	<0.0001	<0.0001	0.0034	0.999	0.0653	<0.0001	<0.0001	0.0014	0.0269	<0.0001	<0.0001	0.9959	0.0074	0.3165	0.8846	0.0001	
Frozen Lb + St vs. Negative control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0086	<0.0001	<0.0001	<0.0001	0.986	0.0002	<0.0001	<0.0001	<0.0001	
Frozen Lb + St + Lr GG vs. Yogurt Lb + St	<0.0001	0.1003	0.0334	<0.0001	<0.0001	<0.0001	<0.0001	0.0453	0.961	<0.0001	0.0652	0.7998	<0.0001	0.9737	0.0002	<0.0001	0.0028	<0.0001	
Frozen Lb + St + Lr GG vs. Yogurt Lb + St + Lr GG	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	0.9645	<0.0001	0.15	<0.0001	<0.0001	0.0295	0.0231	<0.0001	0.9769	0.0005	0.0038	0.2272	<0.0001	
Frozen Lb + St + Lr GG vs. Negative control	0.1432	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0057	0.0095	0.0005	<0.0001	0.9484	<0.0001	<0.0001	<0.0001	<0.0001	0.001 > value > 0.0001
Yogurt Lb + St vs. Yogurt Lb + St + Lr GG	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0003	<0.0001	0.0006	0.0625	0.9994	0.3296	<0.0001	>0.9999	0.9996	0.041	0.4655	<0.0001	0.01 > value > 0.001
Yogurt Lb + St vs. Negative control	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.97	0.0176	<0.0001	>0.9999	0.9084	<0.0001	<0.0001	<0.0001	<0.0001	0.05 > value > 0.01
Yogurt Lb + St + Lr GG vs. Negative control	<0.0001	>0.9999	0.1726	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9977	0.7409	<0.0001	>0.9999	0.7694	<0.0001	<0.0001	<0.0001	<0.0001	>0.05

Supplementary table 3 The table presents the SNPs detected among 60 *L. rhamnosus* GG isolates. From left to right, the columns show the number of isolates in which the SNP was detected, the genomic position of the nucleotide, the alleles and the amino acids they encode, the gene ID and gene product and finally the predictive conclusion based on physio-chemical properties of the mutated amino acid. SNPs at genomic positions 1030390, 2765383, 1611713, 630726 and 877994 were previously identified to represent a sub-population in the strain (Stage, M., Wichmann, A., Jørgensen, M., Vera-jiménez, N. I. & Wielje, M. (2020). *Lactobacillus rhamnosus* GG Genomic and Phenotypic Stability. AEM, 86,6.)

No. of isolates with SNP	Genomic position	Reference nucleotide	Mutated nucleotide	Reference amino acid	Mutated amino acid	Gene ID	Gene product	Amino acid substitution consequence
26	1030390	T	G	Histidine	Glutamine	camS	Lipoprotein	Neutral
26	2765383	G	A	Histidine	Tyrosine	glvA	Maltose-6'-phosphate glucosidase	Neutral
13	2253036	A	G	Aspartic acid	Glycine	CcmA	ABC transporter, multidrug transporter ATPase component	Deleterious
10	1611713	T	C	Glutamine	Arginine	NA	Putative protein without homology	Neutral
5	630729	A	T	Glutamine	Leucine	NA	Conserved protein	Neutral
2	877994	C	T	stop codon	stop codon	ykgB	3-carboxymuconate cyclase	Unchanged stop codon
2	2160010	G	A	Alanine	Threonine	NA	Adenine-specific methyltransferase	Neutral
2	2163622	T	C	Threonine	Alanine	NA	Adenine-specific methyltransferase, Type II restriction enzyme	Neutral
1	1003235	C	A	Glycine	Glycine	NA	Conserved protein	Neutral
1	1664744	G	T	Arginine	Arginine	mdlA	ABC transporter, ATP-binding protein	Deleterious
1	875291	C	A	Glycine	Cysteine	apbE	Thiamine biosynthesis lipoprotein	Deleterious
1	2722438	G	A	Glycine	Arginine	yniG	Transporter, major facilitator superfamily MFS_1	Deleterious
1	998311	C	T	Glycine	Serine	pepF	Oligoendopeptidase F	Deleterious
1	122231	G	T	Glutamic acid	Aspartic acid	dapF	Diaminopimelate epimerase	Neutral
1	2489719	C	A	Alanine	Alanine	cueO	Multicopper oxidase	Neutral
1	2755833	A	G	Alanine	Alanine	NA	Transcriptional regulator, AraC family	Neutral
1	1873089	G	A	Alanine	Valine	NA	Putative protein without homology	Neutral
1	2635941	T	C	Threonine	Alanine	tatD	Mg-dependent Dnase, TatD family	Neutral
1	634973	A	G	Methionine	Threonine	NA	Conserved protein	Neutral
1	1346543	C	T	Arginine	Glutamine	merR	Transcriptional regulator, MerR family	Deleterious
1	910696	G	T	Aspartic acid	Tyrosine	pstS	ABC transporter, phosphate-binding protein	Deleterious