Supplementary Materials: Safety Assessment of Lactobacillus helveticus KLDS1.8701 Based on Whole Genome Sequencing and Oral Toxicity Studies

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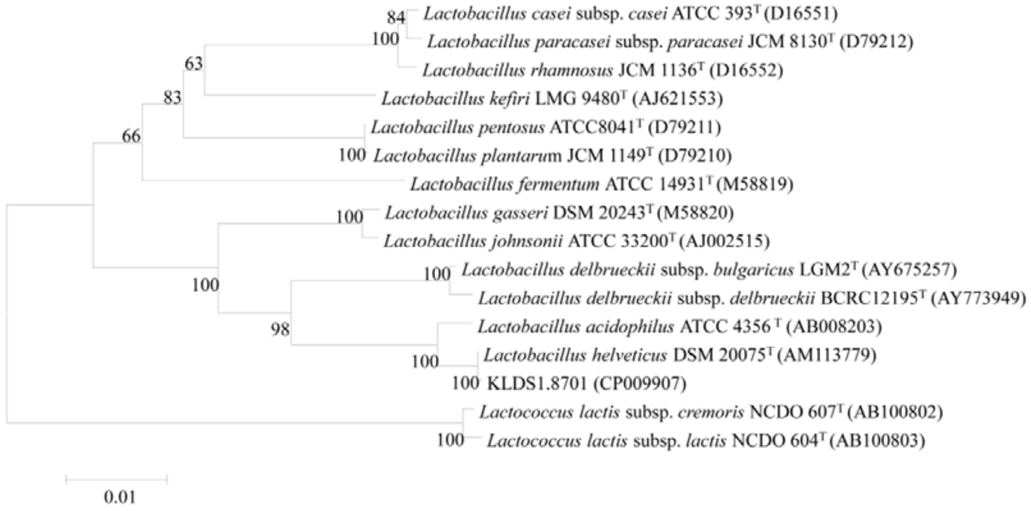

Figure S1 Neighbour-joining tree based on the 16S rRNA gene sequences of strain KLDS1.8701 and phylogenetically related type strains**.** Bootstrap values based on 1000 resampled datasets are shown at branch nodes.

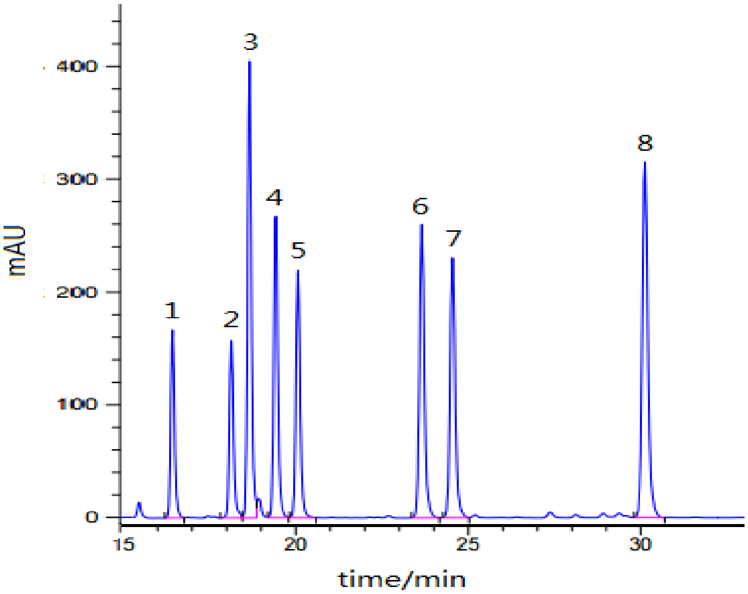

Figure S2 Typical HPLC chromatograms of biogenic amines in standard solution. 1, tryptamine; 2, 2-phenyl – ethylamine; 3, putrescine; 4, cadaverine; 5, histamine; 6, tyramine; 7, spermidine; 8,spermine.

Table S1 General features of the phage regions identified in the *L. helveticus* KLDS1.8701 genome.

Position	Completeness	Total proteins	Most Common Phage	GC percentage
689121-696714	incomplete	13	NC_023719	35.13%
1980240-1988321	incomplete	10	NC_023007	35.61%
2050850-2074701	questionable	16	NC_009904	37.26%

Table S2 General features of the CRISPR loci identified in the *L. helveticus* KLDS1.8701 genome.

Position	Length	Repeat consensus	Repeat length	Spacers number
735863-736129	266 bp	GTTTTCTCCACGTAAGTGGAGGT	23 bp	4
890302-891796	1494 bp	GTATTCTCCACGTATGTGGAGGTGATCC	28 bp	24
2050850-2074701	759 bp	GTATTCTCCACGTATGTGGAGGTGATCC	28 bp	12

Table S3 Putative genes for Type I restriction modification (R/M) system in the *L. helveticus* KLDS1.8701 genome.

Encoded protein	Locus tag(s)
Type I restriction enzyme, R subunit	HUO_RS03985
Type I restriction enzyme M protein	HUO_RS01105
	HUO_RS03980
	HUO_RS03990
	HUO_RS04630
	HUO_RS06070
	HUO_RS08260
Type I restriction enzyme, S subunit	HUO_RS01110
	HUO_RS04635

Table S4 Putative antibiotics resistance genes in the *L. helveticus* KLDS1.8701 genome by searching with the comprehensive antibiotic resistance database (CARD).

Locus	ARO	Associate antibiotics	E-value	Identity	Coverage
HUO_RS00060	ARO:3004045	isoniazid	5.52E-82	47	98
HUO_RS00090	ARO:3003463	isoniazid	6.02E-78	36	92
HUO_RS00235	ARO:3000535	efflux pump complex	8.89E-18	30	74
HUO_RS00240	ARO:3002881	lincosamide	3.28E-20	30	80
HUO_RS00350	ARO:3002832	efflux pump complex	1.94E-24	32	83
HUO_RS00355	ARO:3002833	efflux pump complex	2.40E-18	34	79
HUO_RS00460	ARO:3000535	efflux pump complex	1.48E-52	42	92
HUO_RS00790	ARO:3000124	beta-lactam	1.61E-18	30	79
HUO_RS00875	ARO:3003459	fluoroquinolone	0	47	97
HUO_RS01135	ARO:3000535	efflux pump complex	9.88E-29	32	100
HUO_RS01270	ARO:3002943	vancomycin	3.28E-57	35	83
HUO_RS01440	ARO:3002928	vancomycin	5.92E-58	43	95
HUO_RS01580	ARO:3000535	efflux pump complex	4.61E-29	32	88
HUO_RS01705	ARO:3000535	efflux pump complex	1.19E-39	37	87

HUO_RS01715	ARO:3002909	vancomycin	7.76E-69	36	95
HUO_RS01780	ARO:3000535	efflux pump complex	5.46E-36	38	91
HUO_RS02075	ARO:3003730	mupirocin	1.30E-07	30	70
HUO_RS02165	ARO:3003775	fosfomycin	7.56E-98	40	99
HUO_RS02325	ARO:3002972	vancomycin	1.48E-46	31	83
HUO_RS02630	ARO:3003950	efflux pump complex	2.46E-25	31	84
HUO_RS02635	ARO:3003748	efflux pump complex	2.72E-27	30	87
HUO_RS03565	ARO:3003730	mupirocin	1.15E-09	32	78
HUO_RS03870	ARO:3002987	efflux pump complex	3.25E-30	30	99
HUO_RS04155	ARO:3000838	efflux pump complex	1.00E-80	51	97
HUO_RS04160	ARO:3000839	efflux pump complex	1.06E-74	34	80
HUO_RS04895	ARO:3002818	efflux pump complex	3.08E-37	49	86
HUO_RS05070	ARO:3002985	polymyxin	2.61E-44	35	80
HUO_RS05975	ARO:3003294	fluoroquinolone	2.39E-157	35	96
HUO_RS05980	ARO:3003459	fluoroquinolone	0	44	96
HUO_RS06105	ARO:3000535	efflux pump complex	3.52E-34	34	89
HUO_RS06510	ARO:3000300	dalfopristin	4.42E-157	45	99
HUO_RS07130	ARO:3003950	efflux pump complex	1.25E-25	31	78
HUO_RS07330	ARO:3002857	trimethoprim	5.05E-33	34	95
HUO_RS07575	ARO:3003359	elfamycin	0	68	98
HUO_RS07860	ARO:3003578	polymyxin	7.76E-43	31	99
HUO_RS08210	ARO:3002963	vancomycin	1.44E-13	31	86
HUO_RS08230	ARO:3002925	vancomycin	4.27E-54	40	97
HUO_RS09145	ARO:3000535	efflux pump complex	8.14E-42	36	90
HUO_RS09155	ARO:3002882	lincosamide	0	48	99
HUO_RS09160	ARO:3002881	lincosamide	0	48	96
HUO_RS09940	ARO:3000535	efflux pump complex	5.28E-35	36	97
HUO_RS09970	ARO:3000535	efflux pump complex	6.79E-51	41	86
HUO_RS10220	ARO:3003986	efflux pump complex	2.40E-72	30	99
HUO_RS10270	ARO:3002987	efflux pump complex	3.29E-22	31	96
HUO_RS10320	ARO:3000575	vancomycin	9.78E-08	33	78
HUO_RS10355	ARO:3002925	vancomycin	1.96E-68	48	99
HUO_RS10360	ARO:3002882	lincosamide	3.51E-106	34	86
HUO_RS10365	ARO:3002881	lincosamide	1.89E-108	34	90
HUO_RS10400	ARO:3003470	aminoglycoside	1.97E-24	30	77
HUO_RS10460	ARO:3000535	efflux pump complex	3.51E-46	39	98

Table S5 Putative virulence factors in the *L. helveticus* KLDS1.8701 genome by searching with the virulence factor database (VFDB).

Locus tag(s)	VFDB_ID	VFDB_Genes
HUO_RS03090	VFG012103	(groEL) chaperonin GroEL
HUO_RS01440	VFG006826	(lisR) two-component response regulator
HUO_RS02290	VFG005363	(sspA) surface protein D
HUO_RS06430	VFG037112	(msrA/B(pilB)) peptide methionine sulfoxide reductase msrA/msrB
HUO_RS05430	VFG043573	(CT396) molecular chaperone DnaK
HUO_RS05615	VFG026967	(sigA/rpoV) RNA polymerase sigma factor rpoD
HUO_RS02030	VFG005801	(alp2) alpha-like protein
HUO_RS07575	VFG016490	(tuf) translation elongation factor Tu
HUO_RS08570	VFG000077	(clpP) ATP-dependent Clp protease proteolytic subunit
HUO_RS06800	VFG000080	(clpE) ATP-dependent protease
HUO_RS08890	VFG005871	(hasC) UTP--glucose-1-phosphate uridylyltransferase
HUO_RS10015	VFG005865	(SMU.322c) glucose-1-phosphate uridylyltransferase

Table S6 Body weight and food data of male and female rats after oral administration of *L. helveticus* KLDS1.8701 for 28 days.

Index	Males			Females		
Treatment	Control	Low	High	Control	Low	High
Body weight gain (g)	69.9±6.7	72.6±7.1	67.1±6.1	32.0±2.7	30.5±2.1	28.4±3.1
Daily Food consumption (g)	24.1±0.9	23.7±1.3	23.1±1.8	16.9±1.2	16.8±1.1	16.5±1.7
Food Efficiency	0.104±0.011	0.109±0.007	0.101±0.003	0.068±0.006	0.069±0.006	0.065±0.008

Values are presented as mean ± standard deviation (n=6). Control, sterile normal saline; Low, 1 × 10^9 CFU of *L. helveticus* KLDS1.8701/kg BW; High, 1 × 10^{10} CFU of *L. helveticus* KLDS1.8701/kg BW.

Table S7 Relative organ weights (%) of male and female rats after oral administration of *L. helveticus* KLDS1.8701 for 28 days.

Organs	Males			Females		
Treatment	Control	Low	High	Control	Low	High
Heart	0.335±0.038	0.300±0.049	0.321±0.136	0.307±0.033	0.299±0.027	0.296±0.002
Liver	2.898±0.361	2.931±0.254	3.055±0.965	3.067±0.277	3.489±0.580	2.865±0.277
Spleen	0.164±0.047	0.178±0.029	0.183±0.036	0.204±0.052	0.195±0.040	0.215±0.030
Lung	0.796±0.143	0.811±0.162	0.841±0.078	0.636±0.092	0.640±0.163	0.669±0.058
Kidney	0.368±0.032	0.332±0.018	0.341±0.044	0.313±0.026	0.308±0.030	0.302±0.017
Brain	0.562±0.037	0.610±0.052	0.591±0.033	0.720±0.065	0.701±0.043	0.713±0.056
Adrenal	0.021±0.004	0.019±0.002	0.018±0.004	0.028±0.002	0.031±0.004	0.031±0.003
Thymus	0.027±0.007	0.029±0.017	0.031±0.005	0.023±0.007	0.021±0.004	0.020±0.011
Testes	0.476±0.052	0.480±0.021	0.468±0.085	-	-	-
Epididymides	0.322±0.017	0.328±0.053	0.341±0.040	-	-	-
Uterus	-	-	-	0.242±0.053	0.250±0.041	0.237±0.045
Ovary	-	-	-	0.062±0.007	0.053±0.006	0.055±0.009

Values are presented as mean ± standard deviation (n=6).Control, sterile normal saline; Low, 1 × 10^9 CFU of *L. helveticus* KLDS1.8701/kg BW; High, 1 × 10^{10} CFU of *L. helveticus* KLDS1.8701/kg BW.

Table S8 β-glucosidase and β-glucuronidase activities in the cecal contents of male and female rats after oral administration of *L. helveticus* KLDS1.8701 for 28 days.

Treatment	**Males**		**Females**	
	β-glucosidase(unit/g)	β-glucuronidase (unit/g)	β-glucosidase(unit/g)	β-glucuronidase (unit/g)
Control	9.40±0.53	3.69±0.39[a]	9.66±0.51	3.73±0.42[a]
Low	9.16±0.45	2.56±0.21[b]	9.48±0.49	2.72±0.28[b]
High	9.27±0.86	2.58±0.39[b]	9.75±0.66	2.59±0.52[b]

Values are presented as mean ± standard deviation (n=6). Significant differences ($P < 0.05$) among different treatments are indicated with different letters (a, b). Control, sterile normal saline; Low, 1×10^9 CFU of *L. helveticus* KLDS1.8701/kg BW; High, 1×10^{10} CFU of *L. helveticus* KLDS1.8701/kg BW.