



# In vitro safety assessments and antimicrobial activities of *Lactobacillus rhamnosus* strains isolated from a fermented mare's milk

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**Table S1. Cell growth of *L. rhamnosus* strains and human fecal bacteria in the basal medium supplemented with or without mucin and glucose as carbon sources.**

Bacteria	Basal medium		+ 0.3% HGM Type III		+ 1% glucose		+ 0.3% HGM Type III and 1% glucose	
	OD <sub>600nm</sub>	pH	OD <sub>600nm</sub>	pH	OD <sub>600nm</sub>	pH	OD <sub>600nm</sub>	pH
FSMM15	0.23 ± 0.17	6.60 ± 0.12	0.40 ± 0.24	6.54 ± 0.08	1.76 ± 0.27*	4.26 ± 0.01	2.04 ± 0.24*	4.32 ± 0.01
FSMM22	0.23 ± 0.07	6.59 ± 0	0.34 ± 0.04	6.49 ± 0.01	4.33 ± 0.05*	3.70 ± 0	3.66 ± 0.22*#	3.72 ± 0.01
FSMM26	0.30 ± 0.04	6.57 ± 0.02	0.34 ± 0.03	6.51 ± 0.01	3.40 ± 0.58*	3.69 ± 0	3.72 ± 0.21*	3.72 ± 0.01
LGG	0.43 ± 0.03	6.56 ± 0.02	0.49 ± 0.07	6.49 ± 0.01	2.69 ± 0.18*	3.98 ± 0.01	2.75 ± 0.05*	3.97 ± 0.01
HFB	2.94 ± 0.18	6.72 ± 0.04	3.79 ± 0.02*	6.47 ± 0.05	2.97 ± 0.02	4.19 ± 0.01	2.88 ± 0.01	4.27 ± 0.09
Autoclaved HFB	0.02 ± 0.01	6.76 ± 0.01	0.02 ± 0.01	6.81 ± 0.05	0.02 ± 0.01	6.47 ± 0.04	0.02 ± 0.00	6.51 ± 0.03
None <sup>a</sup>	–	6.75 ± 0.02	–	6.74 ± 0.02	–	6.48 ± 0.01	–	6.61 ± 0.09

<sup>a</sup>No inoculant as control.

\*Significantly different with the basal medium.

#Significantly different with 1% glucose supplementation.

These values were measured after 48 h of the main cultivation. The data are expressed as mean ± SD from 3 replications.

**Table S2. Degradation rates of carbohydrate and protein contents of the mucin after 48-h incubation with the tested bacteria.**

Bacteria	%Degradation			
	+ 0.3% HGM Type III		+ 0.3% HGM Type III and 1% glucose	
	Carbohydrate	Protein	Carbohydrate	Protein
FSMM15	-14.66 ± 30.07	-25.38 ± 5.57	11.18 ± 20.87	-24.70 ± 11.64
FSMM22	-7.95 ± 8.10	2.38 ± 17.14	15.78 ± 12.60	17.66 ± 30.50
FSMM26	-8.00 ± 8.06	2.29 ± 20.48	-5.88 ± 19.18	-20.18 ± 27.01
LGG	1.69 ± 24.14	5.17 ± 40.03	-1.56 ± 9.72	-22.93 ± 9.24
HFB	87.48 ± 1.10	96.84 ± 1.20	31.99 ± 13.18	19.51 ± 61.11
Autoclaved HFB	0	0	0	0

Carbohydrate and protein recoveries were calculated as describe in Miller & Hoskins (1981) as follows:

$$\% \text{ Degradation} = 1 - (\text{concentration in inoculated culture}) / (\text{concentration in negative control}) \times 100.$$

According to this equation, one which gave more than 25% Degradation of carbohydrate or 30% Degradation of protein was defined as giving actual mucus degradation activity.

**Table S3. pH sensitivities of the pathogenic bacteria used in this study.**

Enteropathogenic bacteria	Inhibition activity of adjusted MRS medium (diameter in mm)						
	pH 7.0	pH 6.6	pH 5.0	pH 4.3	pH 4.0	pH 3.0	pH 2.0
<i>S. Typhimurium</i> LT-2	0	0	0	0	8.67 ± 1.15	12.33 ± 1.53	13.50 ± 1.32
<i>L. monocytogenes</i>	0	0	0	0	8.67 ± 1.15	12.33 ± 1.53	13.67 ± 1.15
MRSA	0	0	0	0	0	0	9.67 ± 0.58
MSSA	0	0	0	0	0	0	10.67 ± 0.58
<i>E. coli</i> O157	0	0	0	0	0	9.00 ± 0	10.50 ± 0
<i>S. sonnei</i>	0	0	0	0	0	10.00 ± 0	11.50 ± 0

To check the effects of pH of the culture medium on the antimicrobial activities, MRS broths, whose pH was adjusted to 7.0, 6.6, 5.0, 4.3, 4.0, 3.0, and 2.0 with 0.5 mM HCl, were prepared and freeze dried. By reconstruction of the lyophilized broths, 20 times concentrated solutions were prepared and applied for the disc diffusion assay as described in the text.