Supplementary Files to

Lacticaseibacillus rhamnosus impedes growth of *Listeria* spp. in cottage cheese through manganese limitation

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Table S1. Composition of manganese-deficient CDM-a and CDM-b. All components were dissolved in MilliQ water as various stock solutions and mixed, after which the pH was set to 6.8 with KOH and the volume was adjusted to 1 L and filter-sterilized.

Component	CDM-a	CDM-b
Basal components		
Glucose	27.76 mM	27.76 mM
Sodium acetate	15.24 mM	12.19 mM
Ammonium citrate	2.47 mM	2.47 mM
K_2HPO_4	5.74 mM	14.35 mM
KH_2PO_4	36.74 mM	22.04 mM
$NaHCO_3$	-	4.83 mM
Urea	-	2 mM
Amino acids		
L-Alanine	3.4 mM	0.90 mM
L-Arginine	1.1 mM	0.46 mM
L-Asparagine	0.8 mM	0.61 mM
L-Aspartate	0.8 mM	0.60 mM
L-Glutamate	2.1 mM	0.55 mM
L-Glutamine	0.7 mM	0.55 mM
Glycine	2.7 mM	1 .07 mM
L-Histidine	0.3 mM	0.52 mM
L-Isoleucine	0.8 mM	0.61 mM
L-Leucine	0.8 mM	0.61 mM
L-Lysine	1.4 mM	0.55 mM
L-Methionine	0.7 mM	0.54 mM
L-Phenylalanine	1.2 mM	0.48 mM
L-Proline	2.6 mM	0.70 mM
L-Serine	2.8 mM	0.76 mM
L-Threonine	1.7 mM	0.67 mM
L-Tryptophan	0.5 mM	0.39 mM
L-Tyrosine	0.3 mM	0.44 mM
L-Valine	0.9 mM	0.68 mM
Cysteine	0.8 mM	4.13 mM

Component	CDM-a	CDM-b
Vitamins		
Biotin	0.41 µM	0.82 µM
Folic acid	2.27 µM	0.45 µM
Pyridoxal·HCl	9.82 µM	4.91 µM
Riboflavin	2.66 µM	1.33 µM
Thiamine·HCl	2.96 µM	1.48 µM
Nicotinamide	8.19 µM	4.46 µM
Cyanocobalamin	-	0.37 µM
p-Aminobenzoic acid	-	3.65 µM
Ca-pantothenate	2.10 µM	22.67 µM
Lipoic acid	9.69 µM	2.42 µM
L-ascorbic acid	2.84 mM	-
Biotin	0.41 µM	0.82 µM
Folic acid	2.27 µM	0.45 µM
Pyridoxal·HCl	9.82 µM	4.91 µM
Riboflavin	2.66 µM	1.33 µM
Thiamine·HCl	2.96 µM	1.48 µM
Nicotinamide	8.19 µM	4.46 µM
Bases		
Adenine	-	74.00 µM
Guanine	-	66.17 µM
Uracil	-	89.21 µM
Xanthine	-	65.74 µM
Metals		
$MgCl_2 \cdot 6H_2O$	0.98 mM	0.98 mM
$CaCl_2 \cdot 2H_2O$	0.34 mM	0.34 mM
$ZnSO_4 \cdot 7H_2O$	17.39 µM	17.39 µM
$CoSO_4 \cdot 7H_2O$	8.89 µM	8.89 µM
$CuSO_4 \cdot 5H_2O$	10.01 µM	10.01 µM
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	2.02 µM	2.02 µM
$FeCl_3 \cdot 6H_2O$	5.55 µM	5.55 µM
$FeCl_2 \cdot 4H_2O$	12.58 µM	12.58 µM

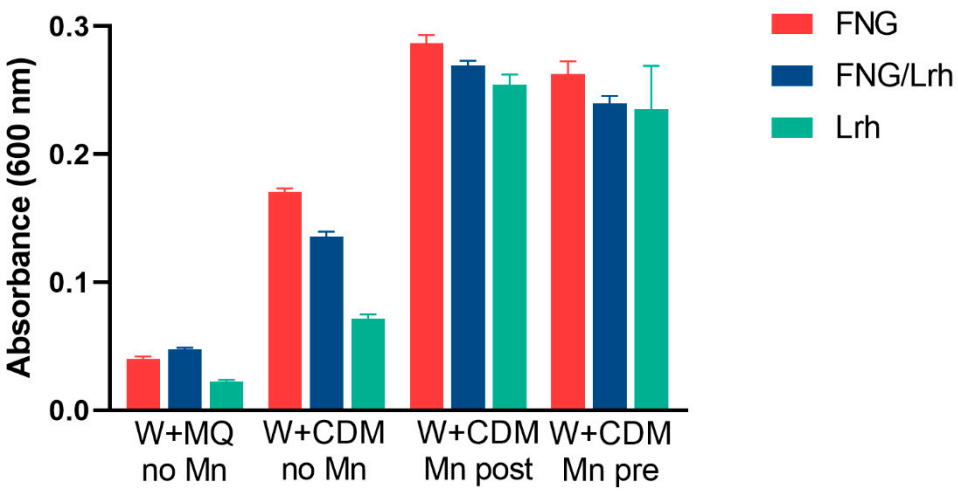


Figure S1. Culture density of *L. innocua* grown for 20 hr in mixtures containing filter-sterilized whey of milk fermented with FNG, Lrh-FQ or both. *L. innocua* BL 86-26 was grown in a pH-adjusted whey/MilliQ mixture (W+MQ, no Mn), a whey/CDM-a mixture (W+CDM, no Mn), a whey/CDM-a mixture supplemented with 6 mg/L $MnCl_2$ (W+CDM, Mn post) or a whey/CDM-a mixture using whey obtained for milk to which 6 mg/L $MnCl_2$ was added prior to fermentation (W+CDM, Mn pre). The whey was mixed in a 1-to-1 ratio with MilliQ or CDM, adjusted to a—for *L. innocua*—growth permissible pH of 6.0, filter sterilized, provided with 0.5% glucose, and inoculated with *L. innocua.* Incubation was carried out in a 96-well plate for 20 hr at 30°C. Shown are averaged absorbance values of pentaplicate measurements corrected using background values of blank medium controls.

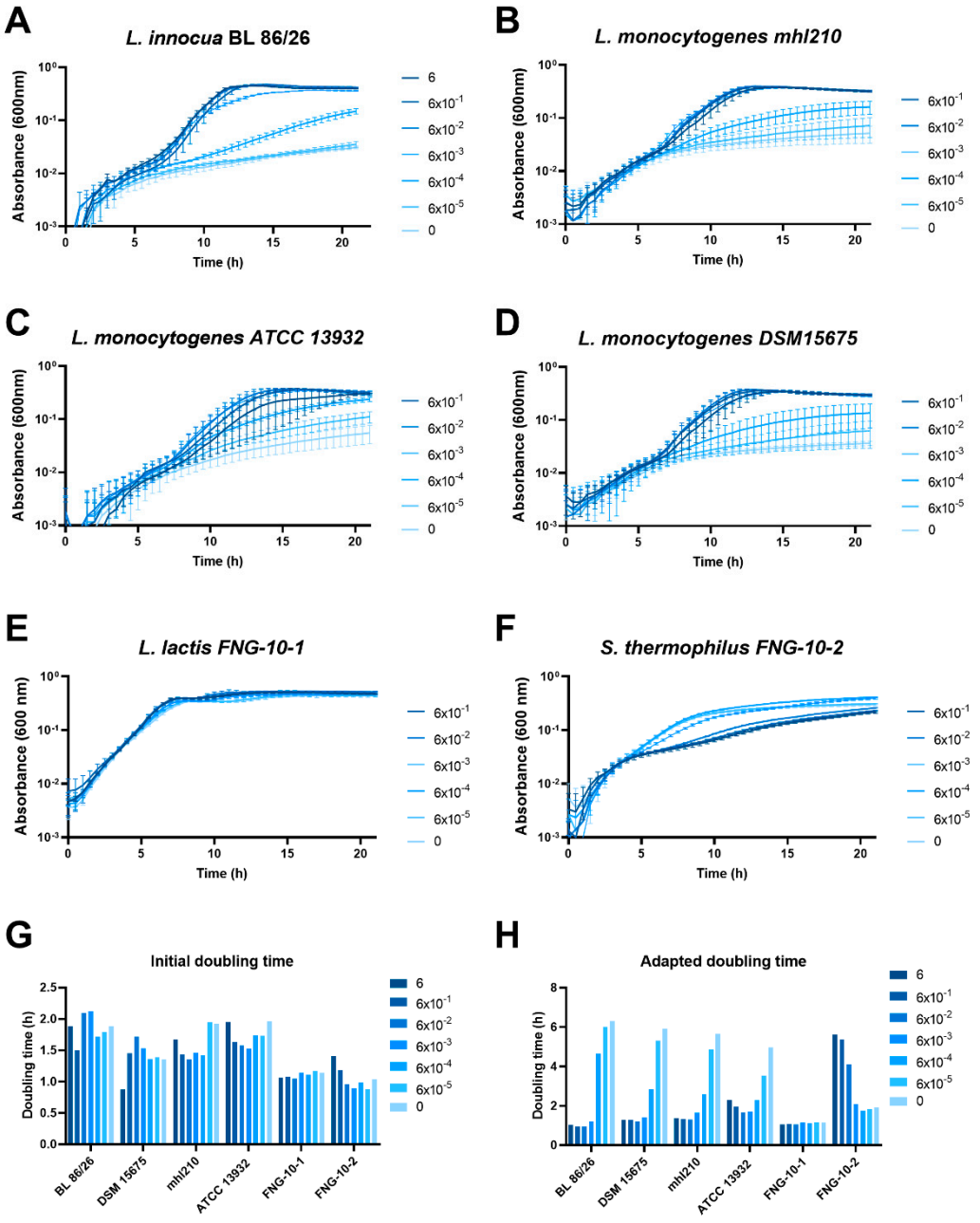


Figure S2. The effect of Mn on the growth rate of various *Listeria* strains and two individual strains obtained from the FNG-10 starter culture. (A-F) Growth curves of Li = *L. innocua*, Lm = *L. monocytogenes*, Ll = *L. lactis*, St = *S. thermophilus* in in Mn-free CDM supplemented with indicated $MnCl_2$ concentration (in mg/L). Depicted are corrected and averaged OD_{600} values. All cultures were grown as standing 200-µL cultures 30°C in six-fold in 96-well plates covered with a transparent seal. Li, Lm, and Ll were grown in CDM-a, whereas St was grown in CDM-b. **(G)** Doubling times calculated over the initial period of growth during which cells pre-grown in in rich nutrient broth are adapting to CDM from pre-growth (derived from the 0–to–7 hr timepoints for *Listeria* strains, and from 0–to–3 hr timepoints for FNG strains). (**H**) Doubling times calculated after adaptation to manganese-depleted CDM (derived from >7 hr or >3 hr timepoints during exponential growth phase for *Listeria* strains or FNG strains, respectively).