

Opposing effect of *Lactobacillus* on *in vitro* *Klebsiella pneumoniae* in biofilm and in an *in vivo* intestinal colonisation model

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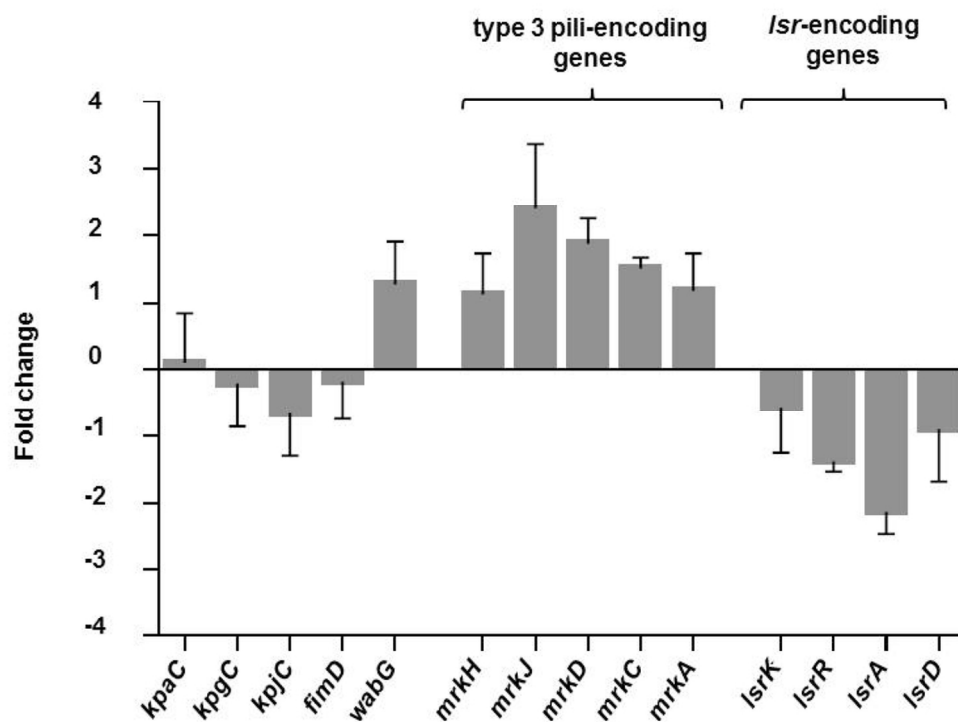
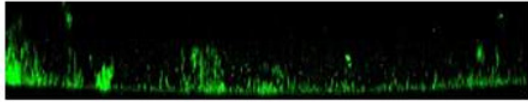
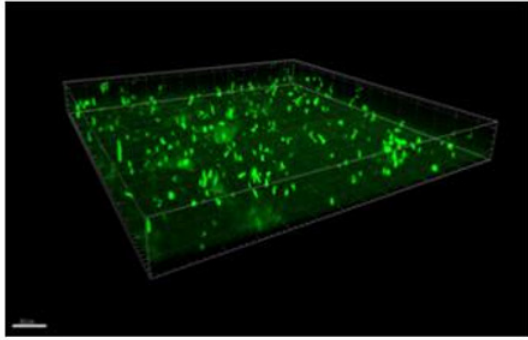
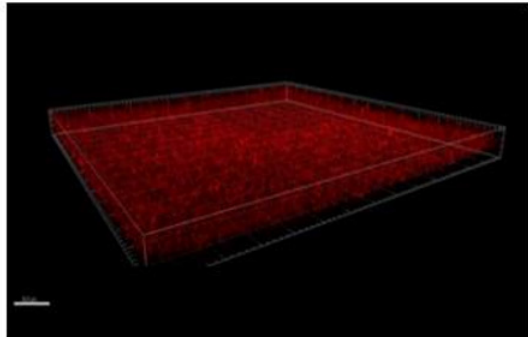


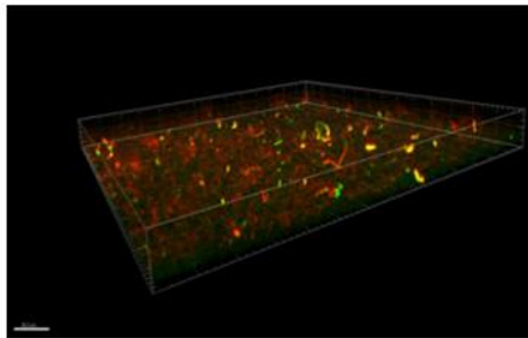
Figure S1. Transcriptional analysis of *Klebsiella pneumoniae* biofilm-related genes. Results are expressed as fold-change, \pm SEM (n=3), of biofilm-related genes expression of *K. pneumoniae* LM21 cultured 8h in mixed biofilm with *Lactobacillus plantarum* CIRM653.



K. pneumoniae LM21
single species biofilm



L. plantarum CIRM653
single species biofilm



K. pneumoniae LM21 +
L. plantarum CIRM653 mixed biofilm

Figure S2. *Lactobacillus plantarum* CIRM653 anti-biofilm activity against *Klebsiella pneumoniae* in continuous flow conditions. Confocal observations of monospecies and mixed biofilms under flow conditions after 8 h of incubation. Gram-negative (*K. pneumoniae* LM21) and Gram-positive (*L. plantarum* CIRM653) bacteria were labelled by green fluorescent SYTO® 9 stain and only Gram-positive bacteria were labelled by red fluorescent hexidium iodide stain.

Table S1. Ranking position from the less effective to the most effective supernatant. Position numbers written in bold represent strains with significant anti-biofilm activity.

Organism	Strain	Origin	<i>K. pneumoniae</i> CH1034		
			Ranking position	biofilm biomass	SEM
<i>L. acidipiscis</i>	DSM 15836	Food	53	77,69%	4,65%
<i>L. acidophilus</i>	ATCC 4356	Human	77	67,53%	2,80%
<i>L. acidophilus</i>	CH537	Unknown	72	69,96%	3,26%
<i>L. acidophilus</i>	CH958	Unknown	43	80,46%	8,78%
<i>L. acidophilus</i>	ATCC 9224	Unknown	4	108,62%	8,66%
<i>L. agilis</i>	DSM 20509	Environment	126	32,28%	0,88%
<i>L. apodemi</i>	DSM 16634	Animal	62	74,39%	6,94%
<i>L. brevis</i>	LBR01	Unknown	38	82,45%	4,85%
<i>L. brevis</i>	ATCC 14869	Human	28	86,50%	10,13%
<i>L. brevisimilis</i>	DSM 6265	Food	55	77,43%	6,28%
<i>L. buchneri</i>	DSM 20057	Food	52	77,87%	8,56%
<i>L. bulgaricus</i>	DSMZ 20081	Food	136	25,73%	0,56%
<i>L. bulgaricus</i>	LB340	Food	123	36,51%	1,87%
<i>L. bulgaricus</i>	LB04	Unknown	64	73,61%	11,78%
<i>L. bulgaricus</i>	ATCC 11842	Food	3	109,27%	7,19%
<i>L. bulgaricus</i>	LB08	Unknown	113	45,45%	7,25%
<i>L. bulgaricus</i>	LB13	Unknown	66	72,16%	8,42%
<i>L. casei</i>	CH956	Unknown	107	48,47%	10,99%
<i>L. casei</i>	LMG 9191 T	Unknown	94	56,86%	19,57%
<i>L. casei</i>	5614	Unknown	37	82,46%	2,60%
<i>L. casei</i>	shirota (yakult)	Unknown	87	62,19%	4,22%
<i>L. casei</i>	CNRZ 1244	Food	5	105,93%	5,04%
<i>L. casei</i>	CNRZ 1874	Unknown	16	92,50%	2,57%
<i>L. casei</i>	BL23	Unknown	112	45,95%	4,80%
<i>L. casei</i>	DN 114001	Unknown	115	44,94%	3,09%
<i>L. casei var rhamnosus</i>	CH086	Unknown	78	67,31%	7,60%
<i>L. casei var rhamnosus</i>	CH960	Unknown	82	63,85%	10,99%

<i>L. casei var rhamnosus</i>	CH495	Unknown	110	47,64%	2,39%
<i>L. casei var rhamnosus</i>	CH526	Unknown	97	55,39%	11,02%
<i>L. casei var rhamnosus</i>	CH528	Unknown	92	59,75%	11,13%
<i>L. coryneformis</i>	DSM 20001	Environment	34	83,13%	10,89%
<i>L. coryneformis</i>	DSM 20004	Environment	42	80,89%	18,99%
<i>L. crispatus</i>	ATCC 33820	Human	8	101,90%	2,04%
<i>L. crispatus</i>	LCR07	Unknown	1	113,44%	18,20%
<i>L. crispatus</i>	LS12	Unknown	65	72,52%	7,36%
<i>L. crispatus</i>	BLL 2005	Unknown	57	77,09%	5,50%
<i>L. curvatus</i>	CNRZ 1335	Food	69	70,26%	10,28%
<i>L. curvatus</i>	CNRZ 1336	Food	26	86,58%	3,80%
<i>L. curvatus</i>	CNRZ 1883	Unknown	41	81,22%	4,81%
<i>L. delbrueckii</i>	CNRZ 208	Food	131	27,17%	5,28%
<i>L. delbrueckii</i>	CIRM 1184	Unknown	79	66,55%	5,04%
<i>L. delbrueckii</i>	CNRZ 448	Unknown	44	79,15%	3,64%
<i>L. delbrueckii</i>	CNRZ 33	Food	130	29,16%	3,48%
<i>L. delbrueckii</i>	CIRM 268	Unknown	135	25,99%	4,04%
<i>L. delbrueckii</i>	CRL 1290	Unknown	46	79,07%	3,92%
<i>L. delbrueckii</i>	CNRZ 396	Unknown	89	61,96%	2,74%
<i>L. delbrueckii</i>	CNRZ 326	Food	58	76,50%	3,05%
<i>L. delbrueckii</i>	CNRZ 333	Food	61	74,70%	10,87%
<i>L. delbrueckii</i>	CIRM 267	Food	127	31,37%	5,35%
<i>L. delbrueckii</i>	CRL 1333	Unknown	36	82,47%	1,61%
<i>L. delbrueckii</i>	CNRZ 1055	Food	9	101,54%	14,38%
<i>L. delbrueckii</i>	CNRZ 226	Unknown	30	85,49%	14,59%
<i>L. delbrueckii</i>	CNRZ 449	Food	50	78,30%	12,44%
<i>L. delbrueckii bulgaricus</i>	CH959	Unknown	15	94,18%	15,36%
<i>L. delbrueckii indicus</i>	DSM 15996	Food	40	82,04%	4,35%
<i>L. diolivorans</i>	DSM 14421	Environment	21	87,82%	11,84%
<i>L. fermentum</i>	LF04	Unknown	83	63,06%	4,66%
<i>L. fermentum</i>	CNRZ 64	Human	59	76,34%	3,73%
<i>L. fermentum</i>	ME3	Human	33	83,94%	20,02%
<i>L. fermentum</i>	CNRZ 209	Food	22	87,71%	10,94%

<i>L. fuchuensis</i>	DSM 14340	Food	14	94,44%	8,52%
<i>L. gasseri</i>	ATCC 33323	Human	71	69,96%	14,16%
<i>L. gasseri</i>	LG11	Unknown	84	62,91%	5,87%
<i>L. gasseri</i>	CNRZ 1943	Unknown	10	99,88%	3,23%
<i>L. gasseri</i>	CNRZ 1944	Human	2	109,54%	11,23%
<i>L. gasseri</i>	CRL 1252	Unknown	19	89,05%	1,71%
<i>L. gasseri</i>	CRL 1311	Unknown	29	85,55%	6,80%
<i>L. halotolerans</i>	DSM 20190	Food	45	79,08%	6,30%
<i>L. harbinensis</i>	DSM 16991	Food	49	78,42%	8,94%
<i>L. helveticus</i>	CNRZ 240	Food	63	74,16%	10,67%
<i>L. helveticus</i>	LH37	Unknown	86	62,27%	7,58%
<i>L. helveticus</i>	IL430	Food	18	90,39%	10,65%
<i>L. homohiochii</i>	DSM 20571	Unknown	140	21,42%	3,53%
<i>L. ingluviei</i>	DSM 15946	Animal	23	87,62%	12,89%
<i>L. intestinalis</i>	CNRZ 219	Unknown	32	84,18%	4,76%
<i>L. johnsonii</i>	CNRZ 1939	Unknown	56	77,35%	11,78%
<i>L. johnsonii</i>	CNRZ 1937	Human	35	82,58%	8,44%
<i>L. johnsonii</i>	CNRZ 1941	Unknown	27	86,54%	2,85%
<i>L. johnsonii</i>	CNRZ 218	Human	109	47,81%	4,01%
<i>L. johnsonii</i>	LCR11	Unknown	98	54,40%	5,71%
<i>L. kunkeei</i>	DSM 12361	Food	52	77,87%	8,56%
<i>L. lactis</i>	LL01	Unknown	80	65,81%	5,79%
<i>L. lactis</i>	LL10	Unknown	81	64,69%	3,42%
<i>L. mucosae</i>	DSM 13345	Animal	134	26,89%	1,69%
<i>L. murinus</i>	CNRZ 220	Animal	137	24,99%	3,06%
<i>L. pantheris</i>	DSM 15945	Animal	93	58,27%	6,71%
<i>L. paracasei</i>	ATCC 334 *	Food	119	41,55%	1,96%
<i>L. paracasei</i>	CIRM 2243	Unknown	17	91,32%	2,75%
<i>L. paracasei</i>	CIRM 1180	Unknown	31	84,27%	9,11%
<i>L. paracasei</i>	CNRZ 318	Unknown	111	46,46%	4,93%
<i>L. paracasei</i>	CNRZ 1328	Unknown	122	45,95%	4,80%
<i>L. paracasei</i>	LPC41	Unknown	75	68,57%	4,00%
<i>L. paracasei</i>	LPC42	Unknown	118	43,42%	5,12%

<i>L. paracasei</i>	Lpc37	Food	100	52,53%	2,30%
<i>L. paracasei ssp tolerans</i>	DSM 20258	Food	39	82,14%	11,44%
<i>L. paralimentarius</i>	DSM 13238	Food	60	75,26%	3,28%
<i>L. paraplantarum</i>	CNRZ 1885	Food	85	62,32%	15,73%
<i>L. paraplantarum</i>	LPP01	Unknown	67	71,59%	6,40%
<i>L. pentosus</i>	NCDO 363	Unknown	73	68,88%	9,78%
<i>L. pentosus</i>	CNRZ 1218	Food	121	40,76%	2,27%
<i>L. pentosus</i>	CNRZ 2268	Unknown	47	79,06%	15,74%
<i>L. plantarum</i>	ATCC BAA-793	Human	99	54,16%	4,86%
<i>L. plantarum</i>	ATCC 14917	Food	125	36,30%	1,19%
<i>L. plantarum</i>	DSM 20174	Food	101	52,42%	2,89%
<i>L. plantarum</i>	DSM 13273	Food	124	36,45%	4,18%
<i>L. plantarum</i>	CIRM 1997	Food	24	87,25%	5,29%
<i>L. plantarum</i>	CIRM 653	Food	129	29,87%	2,80%
<i>L. plantarum</i>	CIRM 2048	Unknown	13	95,09%	6,73%
<i>L. plantarum</i>	LP22	Unknown	128	30,26%	2,87%
<i>L. plantarum</i>	ATCC 14431	Environment	117	43,59%	2,37%
<i>L. plantarum</i>	CNRZ 1571	Unknown	95	56,35%	6,73%
<i>L. plantarum</i>	CNRZ 73	Unknown	20	88,53%	5,67%
<i>L. plantarum argenteratensis</i>	DSM 16365	Food	54	77,55%	6,80%
<i>L. reuteri</i>	ATCC 23272	Human	133	27,08%	25,06%
<i>L. reuteri</i>	CH953	Unknown	74	68,82%	2,63%
<i>L. reuteri</i>	CIRM 251	Unknown	12	96,01%	7,25%
<i>L. rhamnosus</i>	Lcr35	Human	116	44,37%	4,06%
<i>L. rhamnosus</i>	CH962	Unknown	120	41,17%	0,98%
<i>L. rhamnosus</i>	DSMZ 20021	Unknown	70	70,22%	12,45%
<i>L. rhamnosus</i>	LRH04	Unknown	114	45,45%	2,39%
<i>L. rhamnosus</i>	CNRZ 317	Unknown	108	47,88%	3,98%
<i>L. rhamnosus GG</i>	ATCC53103	Human	76	67,83%	6,36%
<i>L. sakei</i>	23k	Food	91	59,79%	3,18%
<i>L. sakei</i>	LSK05	Unknown	103	50,87%	3,77%
<i>L. sakei</i>	CNRZ 1332	Unknown	7	103,83%	5,84%
<i>L. salivarius</i>	DSM 20492	Human	25	87,23%	18,89%

<i>L. salivarius</i>	LS33	Unknown	96	55,78%	2,25%
<i>L. salivarius</i>	CNRZ 228	Unknown	68	70,45%	19,64%
<i>L. salivarius</i>	CNRZ 227	Human	88	62,18%	16,80%
<i>L. salivarius</i>	LS11	Unknown	11	96,13%	5,86%
<i>L. salivarius</i>	CNRZ 1858	Unknown	6	104,26%	9,06%
<i>L. satsumensis</i>	DSM 16230	Food	105	50,16%	3,10%
<i>L. vaccinostercus</i>	DSM 20605	Unknown	48	78,89%	8,21%
<i>L. vaginalis</i>	LVG01	Unknown	106	49,83%	2,88%
<i>L. viridescens</i>	DSM 20410	Food	138	23,51%	3,36%
<i>L. xylosus (lactococcus lactis)</i>	DSM 20175	Food	139	21,85%	3,00%
<i>L. zeae</i>	CH957	Unknown	102	51,50%	13,12%
<i>L. zeae</i>	DSMZ 20011	Food	90	61,81%	20,57%
<i>L. zeae</i>	CIRM 524	Food	132	27,17%	1,51%
<i>L. zeae</i>	CNRZ 230	Unknown	104	50,28%	3,48%

Table S2. Primers used in this study.

Target gene	Primer's name	Function	Oligonucleotide sequence (5'-3')	PCR product size (bp)
<i>kpaC</i>	Kpa_qPCR_Fw	Usher protein	GCTCCGACGATAAAGAATATAAC	113
	Kpa_qPCR_Rv		CAGGTTGGTGTATTTATCTTCC	
<i>kpgC</i>	Kpg_qPCR_Fw	Usher protein	GATGTCTGACGATGAAATGC	125
	Kpg_qPCR_Rv		CAAAGGCTCTGATGATCACATAC	
<i>kpjC</i>	Kpc_qPCR_Fw	Usher protein	CTGATATCACGTTTGTGACG	121
	Kpc_qPCR_Rv		CGGGAATAAACTTCTCGATC	
<i>fimD</i>	fim_qPCR_Fw	Usher protein	GAAAAACATGGGCTATTTCCG	115
	fim_qPCR_Rv		GGATTTGTTATAGAGAAAAACGC	
<i>mrkA</i>	RT-mrkA-Fw	Major type 3 fimbrial subunit	CTAACTGCCAGGCTGTGAT	118
	RT-mrkA-Rv		CCTGTTGTTGCTGGTTGCG	
<i>mrkC</i>	RT-mrkC-Fw	Usher protein	GTCGATCTGTCGCGCTTTTC	95
	RT-mrkC-Rv		AAATCGTAGCGCCCTTTCCA	
<i>mrkD</i>	RT-mrkD-Fw	Adhesin	AATGTCGATGTGGGTTCCAT	123
	RT-mrkD-Rv		GTTGCCATATCCCGTTTCAC	
<i>mrkH</i>	RT-mrkH-Fw	Transcriptional activator	CGCCATGAGCATGACTTTTA	120
	RT-mrkH-Rv		TTTAAGATTCGGCGTTTTGG	
<i>mrkJ</i>	RT-mrkJ-Fw	Phosphodiesterase	TTTCGAGGTAACCGAAAACG	122
	RT-mrkJ-Rv		TAGCGTTAATCCCGCGTAG	
<i>cpxR</i>	RT-cpxR-Fw	DNA-binding response regulator	AGTTGATGATGACCGGGAGC	201
	RT-cpxR-Rv		GGAGTCTGGTGTCTGACG	
<i>lsrA</i>	RT-lsrA-Fw	Transporter subunit	ATCCTTGATGAACCCACCGC	100
	RT-lsrA-Rv		TAAACACGATCCCCACGTCC	
<i>lsrR</i>	RT-lsrR-Fw	Transcriptional repressor	CGGTCGGCGACATTTTAGGT	101
	RT-lsrR-Rv		TAGCTCATCAAGGGTCACGC	
<i>lsrD</i>	RT-lsrD-Fw	Transmembrane transporter	CTCGGCGGGCCAATATTTA	103
	RT-lsrD-Rv		TGCCGACCATCTGTAAACCC	
<i>lsrK</i>	RT-lsrK-Fw	Kinase	AGCTTCTTTACCGCCTGAC	104
	RT-lsrK-Rv		TCCAGCAGCGAATAGGCATC	
<i>wabG</i>	RT-wabG-Fw	Glycosyltransferase	GTA AATTCAGGCTGGCTCTG	111
	RT-wabG-Rv		CGTTCAGTTGCAGATGACTG	