Supplemental Material

Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces

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Contents:

Table S1: Maximal growth rates and OD_{600} values obtained from growth curves

	max growth rate (days^{-1})	**OD_{600} in stationary phase**
A. johnsonii MF4640	7.6	1.2
A. johnsonii MF4642	8.1	1.3
C. sputi MF4643	1.3	1.0
Corynebacterium MF4645	3.4	1.6
Epilithonimonas MF6392	4.8	2.0
K. rhizophila MF6395	2.6	2.0
Kocuria MF4644	1.8	0.1 [a]
Microbacterium MF4634	3.3	2.3
Micrococcus MF6369	1.3	2.0
P. fluorescens MF6394	11.0	2.1
P. mandelii MF4836	9.2	2.0
P. putida MF6396	6.8	2.0
Psychrobacter MF4641	3.8	1.5
R. erythropolis MF4633	4.3	2.0
R. fascians MF4637	3.6	2.0
Sphingomonas MF4632	1.1	0.4 [a]
L. monocytogenes MF4536	4.5	1.0
L. monocytogenes MF5376	4.6	1.0
L. monocytogenes MF5377	4.5	1.0
L. monocytogenes MF5634	4.5	1.1
L. monocytogenes MF4565	4.6	1.1
L. monocytogenes MF5378	4.4	1.1
L. monocytogenes MF5630	4.5	1.0

[a] The strain did not reach stationary phase in 6 days. The value given is the OD_{600} reached after six days of growth.

Table S2: Putative biofilm-associated genes identified in *Pseudomomonas* sp. strains[a]

Polysaccharide Psl				
Query gene/locus tag		**MF4836**	**MF6394**	**MF6396**
pslA	PA2231	-	MF6394_31135	-
pslB	PA2232	MF4836_12955	MF6394_31140	MF6396_02060
pslC	PA2233	-	MF6394_31145	-
pslD	PA2234	-	MF6394_31150	-
pslE	PA2235	-	MF6394_31155	-
pslF	PA2236	-	MF6394_31160	-
pslG	PA2237	-	MF6394_31165	-
pslH	PA2238	-	MF6394_31170	MF6396_02055
pslI	PA2239	-	MF6394_31175	-
pslJ	PA2240	-	MF6394_31180	-
pslK	PA2241	-	MF6394_31190	-
pslL	PA2242	-	-	-
pslM	PA2243	MF4836_20005	-	MF6396_25180
pslN	PA2244	MF4836_27720	MF6394_16270	MF6396_25885
Polysaccharide Pel				
Query gene/locus tag		**MF4836**	**MF6394**	**MF6396**
pelA	PA3064	MF4836_15930	-	-
pelB	PA3063	MF4836_15935	-	-
pelC	PA3062	MF4836_15940	-	-
pelD	PA3061	MF4836_15945	-	-
pelE	PA3060	MF4836_15950	-	-
pelF	PA3059	MF4836_15955	-	-
pelG	PA3058	MF4836_15960	-	-
Aggregative polysaccharide cellulose, *wss* operon				
Query gene		**MF4836**	**MF6394**	**MF6396**
wssB	-	-	-	MF6396_10305
wssC	-	-	-	MF6396_10310
wssD	-	-	-	MF6396_10315
wssE	-	-	-	MF6396_10320
wssF	-	-	-	MF6396_10325
wssG	-	-	-	MF6396_10330
wssH	-	MF4836_08185	MF6394_04460	MF6396_10335
wssI	-	-	-	MF6396_10340
Capsular polysaccharide alginate				
Query gene/locus tag		**MF4836**	**MF6394**	**MF6396**
algD	PA3540	MF4836_08230	MF6394_04505	MF6396_00635
alg8	PA3541	MF4836_08225	MF6394_04500	MF6396_00630
alg44	PA3542	MF4836_08215	MF6394_04490	MF6396_00625
algK	PA3543	MF4836_08210	MF6394_04485	MF6396_00620
algE	PA3544	MF4836_08205	MF6394_04480	MF6396_00615
algG	PA3545	MF4836_08200	MF6394_04475	MF6396_00610
algX	PA3546	MF4836_08195	MF6394_04470	MF6396_00605
algL	PA3547	MF4836_08190	MF6394_04465	MF6396_00600
algI	PA3548	MF4836_08185	MF6394_04460	MF6396_00595
algJ	PA3549	MF4836_08180	MF6394_04455	MF6396_00590
algF	PA3550	MF4836_08175	MF6394_04450	MF6396_00585
algA	PA3551	MF4836_08170	MF6394_04445	MF6396_00580
LapA adhesin				
Query gene/locus tag		**MF4836**	**MF6394**	**MF6396**
lapA	PP0168	MF4836_17645	MF6394_18190	MF6396_24900
lapB	PP0167	MF4836_17635	MF6394_18180	MF6396_22540
lapC	PP0166	MF4836_17630	MF6394_18175	MF6396_22545
lapD	PP0165	MF4836_17655	MF6394_32135	MF6396_24100
lapG	PP0164	MF4836_17660	MF6394_32130	MF6396_24095

[a] *The gene producing the highest-scoring alignment i each BLASTsearch is listed, and all matches with E-values <10^{-9} were included in the table.*

Table S3: Bactericidal suspension test results

Strain	control log_{10} CFU ml^{-1}	log_{10} reductions; 1% Alkalifoam	log_{10} reductions; 1% DesQA	log_{10} reductions; 1.5% Diverfoam
A. johnsonii MF4640	7.1 7.0 4.9 6.9	5.1 >5.0 2.3 >4.9	>5.1 4.4 >2.9 >4.9	>5.1 >5.0 2.9 >4.9
A. johnsonii sp. MF4642	6.8 6.7 6.8	>4.8 >4.7 >4.8	>4.8 4.2 >4.8	>4.8 4.7 >4.8
C. sputi MF4643	7.0 7.0 7.0	1.6 1.1 1.2	5.0 >5.0 >5.0	>5.0 >5.0 >5.0
Corynebacterium sp. MF4645	7.1 6.9 7.0	1.5 1.3 1.6	5.1 >4.9 >5.0	>5.1 >4.9 >5.0
Epilithonimonas sp. MF6392	6.6 5.4 5.1	>4.6 >3.4 3.1	>4.6 >3.4 >3.1	>4.6 >3.4 >3.1
Kocuria sp. MF4644	7.2 7.2 7.2	3.5 4.4 3.0	5.2 >5.2 >5.2	>5.2 >5.2 >5.2
Kocuria rhizophila MF6395	7.0 6.7 6.3	3.8 >4.7 3.7	4.3 >4.7 >4.3	<5.0 >4.7 >4.3
Microbacterium sp. MF4634	7.0 6.9 6.9	>5.0 >4.9 >4.9	>5.0 >4.9 >4.9	4.5 >4.9 >4.9
Micrococcus sp. MF6393	6.9 6.6 6.6	1.8 1.7 1.6	>4.9 >4.6 >4.6	>4.9 >4.6 >4.6
P. putida MF6396	7.2 7.2 6.9	3.7 3.1 4.9	>5.2 >5.2 >4.9	>5.2 >5.2 >4.9
P. fluorescens MF6394	7.1 7.0 7.0	>5.1 >5.0 4.7	>5.1 5.0 >5.0	>5.1 >5.0 >5.0
P. mandelii MF4836	7.4 7.4 7.3 7.0	>5.4 3.4 >5.3 >5.0	5.4 3.7 >5.3 >5.0	>5.4 3.9 >5.3 >5.0
Psychrobacter sp. MF4641	4.6 7.3 5.2 6.4	? 3.5 >3.2 >4.4	? >5.3 >3.2 >4.4	? >5.3 >3.2 >4.4
R. erythropolis MF4633	7.3 7.1 7.0 7.3	5.3 >5.1 4.5 >5.3	? >5.1 >5.0 >5.3	5.3 >5.1 >5.0 >5.3
R. fascians MF4637	~7.0 6.8 7.6	>5.0 >4.8 >5.6	>5.0 >4.8 >5.6	>5.0 >4.8 >5.6
Sphingomonas sp. MF4632	– 6.9 6.6 6.5	– 4.9 >4.6 3.7	– 3.6 >4.6 >4.5	– >4.9 >4.6 >4.5
L. monocytogenes MF4536	7.2 7.0 7.1	>5.2 >5.0 >5.1	>5.2 >5.0 >5.1	>5.2 >5.0 >5.1
L. monocytogenes MF5376	7.2 7.2 7.1	5.2 >5.2 3.6	>5.2 >5.2 >5.1	>5.2 >5.2 4.4
L. monocytogenes MF5634	7.2 7.1 7.3	5.2 >5.1 >5.3	3.8 >5.1 >5.3	>5.2 >5.1 >5.3
L. monocytogenes MF5377	7.2 7.2 7.2	4.6 >5.2 >5.2	>5.2 >5.2 >5.2	>5.2 >5.2 >5.2
L. monocytogenes MF4565	7.4 7.2 7.3	>5.4 >5.2 4.6	>5.4 >5.2 >5.3	>5.4 >5.2 >5.3
L. monocytogenes MF5630	7.1 7.1 7.1 7.0	>5.1 >5.1 3.3 >5.0	>5.1 >5.1 3.4 >5.0	>5.1 >5.1 3.2 >5.0
L. monocytogenes MF5378	7.3 7.1 7.3 7.1	>5.3 >5.1 3.3 5.1	>5.3 >5.1 ~3.3 >5.1	>5.3 >5.1 ~3.3 >5.1

Table S4: Number of conveyor belt coupons subjected to CLSM imaging [a]

		Before C&D		After C&D	
Front of coupon		H_2O	QAC	QAC	PAA
Multigenera biofilms	Day 4	5 (21)	NA	5 (20)	3 (15)
	Day 7	5 (15)	5 (15)	5 (15)	NT
L. monocytogenes biofilms	Day 4	5 (22)	NA	1 (5)	1 (5)
	Day 7	5 (15)	NT	NT	NT
Back of coupon					
Multigenera biofilms	Day 4	3 (15)	NA	3 (10)	1 (3)
	Day 7	5 (15)	5 (15)	5 (15)	NT
L. monocytogenes biofilms	Day 4	3 (11)	NA	2 (6)	NT
	Day 7	5 (15)	5 (15)	2 (6)	NT

[a] *The total number of Z-scans acquired for each set of coupons is given in parenthesis. NA: not applicable. NT: not tested*

Table S5: GenBank accessions for publicly available sequences used in phylogenetic analyses

Strain (type strain of the species unless strain name is listed)	**Accession numbers for sequences used in phylogenetic analysis**				
	16S rRNA	***gyrB***	***rpoB***	***rpoD***	**WGS**
Pseudomonas aeruginosa	HE978271	AJ633104	AJ717442	AB039607	
P. alkylphenolia KL28					CP009048
P. antarctica	AJ537601	FN554169	FN554727	FN554450	
P. arsenicoxydans	FN645213	HE800469	HE800503	HE800488	
P. aureofaciens	AY509898	FN554172	AJ717426	FN554453	
P. azotoformans	D84009	AB039411	AJ717458	AB039547	
P. corrugata	D84012	AB039460	AJ717487	AB039566	
P. costantinii	AF374472	FN554180	FN554732	FN554461	
P. cremoricolorata	AB060137	FN554181	AJ717476	FN554462	
P. donghuensis					AJJP00000000
P. entomophila	AY907566	AY907567			CT573326
P. extremorientalis	AF405328	FN554182	FN554733	FN554464	
P. fluorescens	D84013	D86016	AJ717451	AB039545	
P. fluorescens A506					CP003041
P. fluorescens FW300-N2E3					CP012830
P. fluorescens Pt14					CP017296
P. fragi	AF094733	FN554184	AJ717444	FN554466	
P. frederiksbergensis	AJ249382	AM084676	AJ717465	AM084335	
P. fulva	AB060136	AB039395	AJ717419	AB039586	
P. gessardii	AF074384	FN554186	AJ717438	FN554468	
P. grimontii	AF268029	FN554188	AJ717439	FN554470	
P. japonica	AB126621	GQ996725	HE577800	HE577795	
P. libanensis	AF057645	FN554195	AJ717454	FN554477	
P. lini	AY035996	FN554196	AJ717466	FN554478	
P. lutea	AY364537	FN554198	FN554738	FN554480	
P. mandelii	AF058286	FN554200	AJ717435	FN554482	
P. marginalis	Z76663	AB039448	AJ717425	AB039575	
P. migulae	AF074383	FN554204	AJ717446	FN554486	
P. monteilii	AF064458	FN554205	AJ717455	FN554488	
P. mosselii	AF072688	FN554207	FN554744	FN554491	
P. orientalis	AF064457	FN554209	AJ717434	FN554493	
P. palleroniana	AY091527	FN554213	FN554747	FN554497	
P. parafulva	AB060132	FN554216	AJ717471	FN554500	
P. plecoglossicida	AB009457	FN554218	AJ717456	FN554503	
P. poae	AJ492829	FN554219	FN554751	FN554504	
P. protegens	AJ278812	HE800482	HE800514		CP003190
P. putida	D84020	AB039451		AB039581	AP013070
P. rhizosphaerae	AY152673	FN554224	FN554755	FN554510	
P. rhodesiae	AF064459	FN554225	AJ717431	FN554511	
P. synxantha	D84025	AB039415	AJ717420	AB039550	
P. syringae	DQ318866	AB039428	FN554759	AB039516	
P. tolaasii	AF255336	AB039423	AJ717467	AB039561	
P. trivialis	AJ492831	FN554230	FN554762	FN554515	
P. veronii	AF064460	FN554233	AJ717445	FN554518	
P. vranovensis	AY970951	HE577791	HE577799	HE577793	
Cellvibrio japonicus Ueda107					CP000934
Acinetobacter johnsonii					APON00000000
A. schindleri					APPQ00000000
A. baylyi					APPT00000000
A. towneri					APPY00000000
A. johnsonii ANC 3681					APPZ00000000
A. bouvetii					APQD00000000
A. baumannii					APRG00000000
A. indicus					AYET00000000
A. lwoffii					AYHO00000000
Psychrobacter sp. PRwf-1					CP000713

Table S6 References used for 16S rRNA amplicon analysis in Qiime

The 17 partial 16S rRNA (V3-V4) gene sequences of the 16 background flora strains plus *L. monocytogenes* used in experiments in the current study. This sequence file was used as the reference database to identify the different taxa in the Qiime analysis.

>P-putida-MF6396
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>P-fluorescens-MF6394
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>Micrococcus-MF6369
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>Epilithonimonas-MF6392
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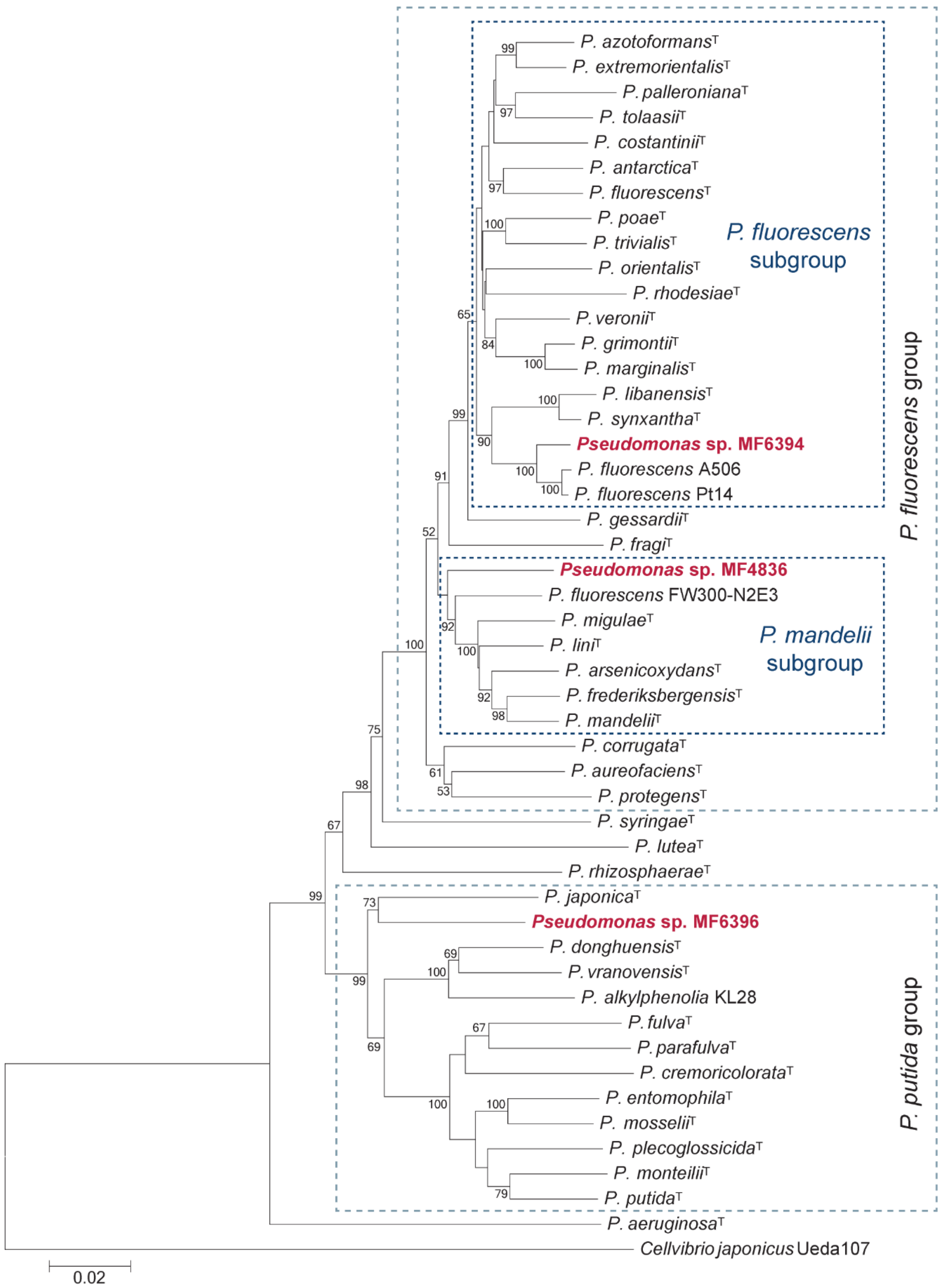

FIG S1

Neighbor-joining phylogeny of strains belonging to the genus *Pseudomonas*. The tree is based on concatenated partial 16S rRNA, *gyrB*, *rpoB*, and *rpoD* gene sequences, and *Cellvirio japonicus* Ueda107 was used as an outgroup. The strains sequenced in the current study are in red bold. Type strains are indicated by superscript T. Evolutionary distances were computed with MEGA (version 7) using the Jukes-Cantor method. The bar indicates the number of base substitutions per site. Percentage bootstrap values above 50% (from 1000 replicates) are shown next to the nodes.

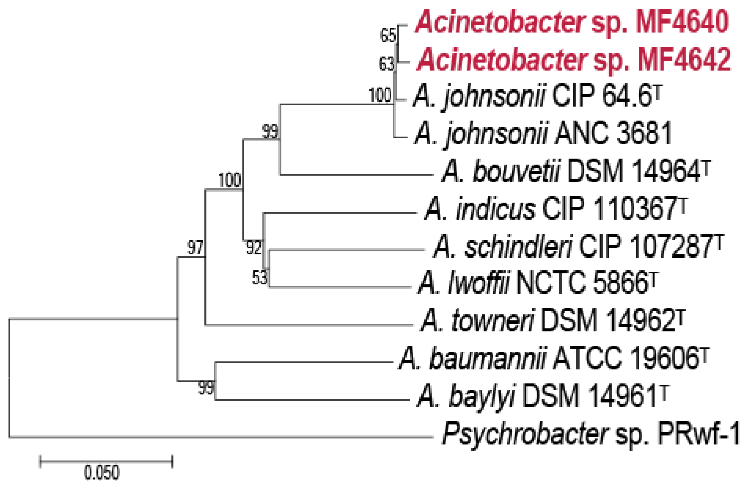

FIG S2

Neighbor-joining phylogeny of strains belonging to the genus *Acinetobacter*. The tree is based on concatenated partial *cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB* and *rpoB* gene sequences according to the MLST scheme described by Diancourt *et al*. (1). *Psychrobacter* sp. PRwf-1 was used as an outgroup. The strains sequenced in the current study are in red bold. Type strains are indicated by superscript T. Evolutionary distances were computed with MEGA (version 7) using the Jukes-Cantor method. The bar indicates the number of base substitutions per site. Percentage bootstrap values above 50% (from 1000 replicates) are shown next to the nodes.

1. **Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S.** 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One **5:**e10034.

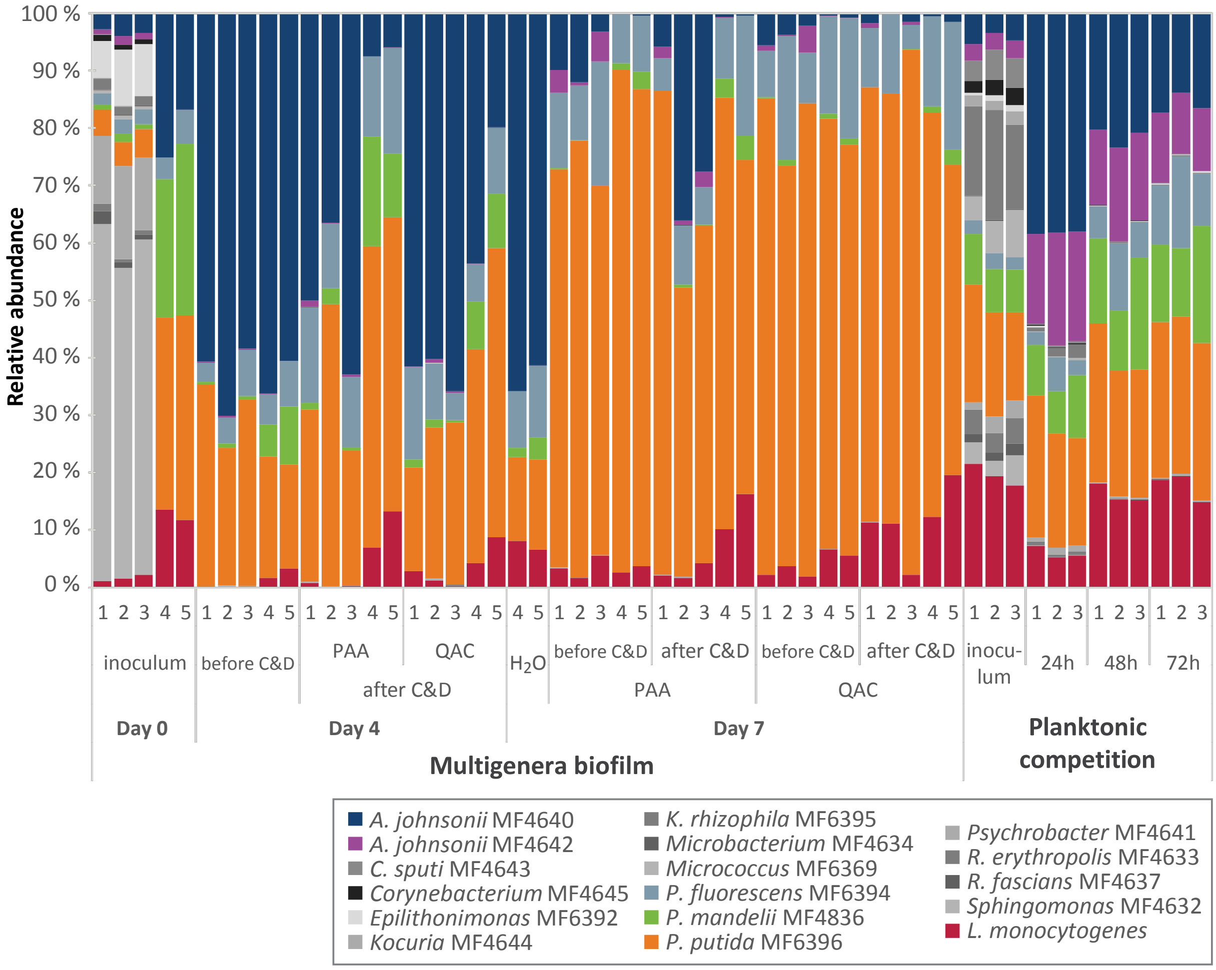

FIG S3

Development of the microbiota in multigenera biofilms and planktonic cultures, showing results from individual experiments. Numbers 1 to 5 below each column refer to experiment number. The frequencies of different bacterial strains was determined by 16S rRNA amplicon sequencing. Multigenera biofilms were grown on conveyor belt coupons, and inoculated with *L. monocytogenes* plus either 16 (experiments 1 to 3) or four (experiments 4 and 5) background microbiota strains. The biofilms were allowed to develop for four days before being subjected to daily cleaning with Alkalifoam and disinfection with either a QAC-based or a PAA-based disinfection agent. Coupons were harvested either before or after C&D on the day of harvest, as indicated. Planktonic competition cultures were inoculated with *L. monocytogenes* plus 16 background microbiota strains and grown with shaking in Erlenmeyer flasks for a total of 72 h. All samples were grown in BHI broth at 12°C.

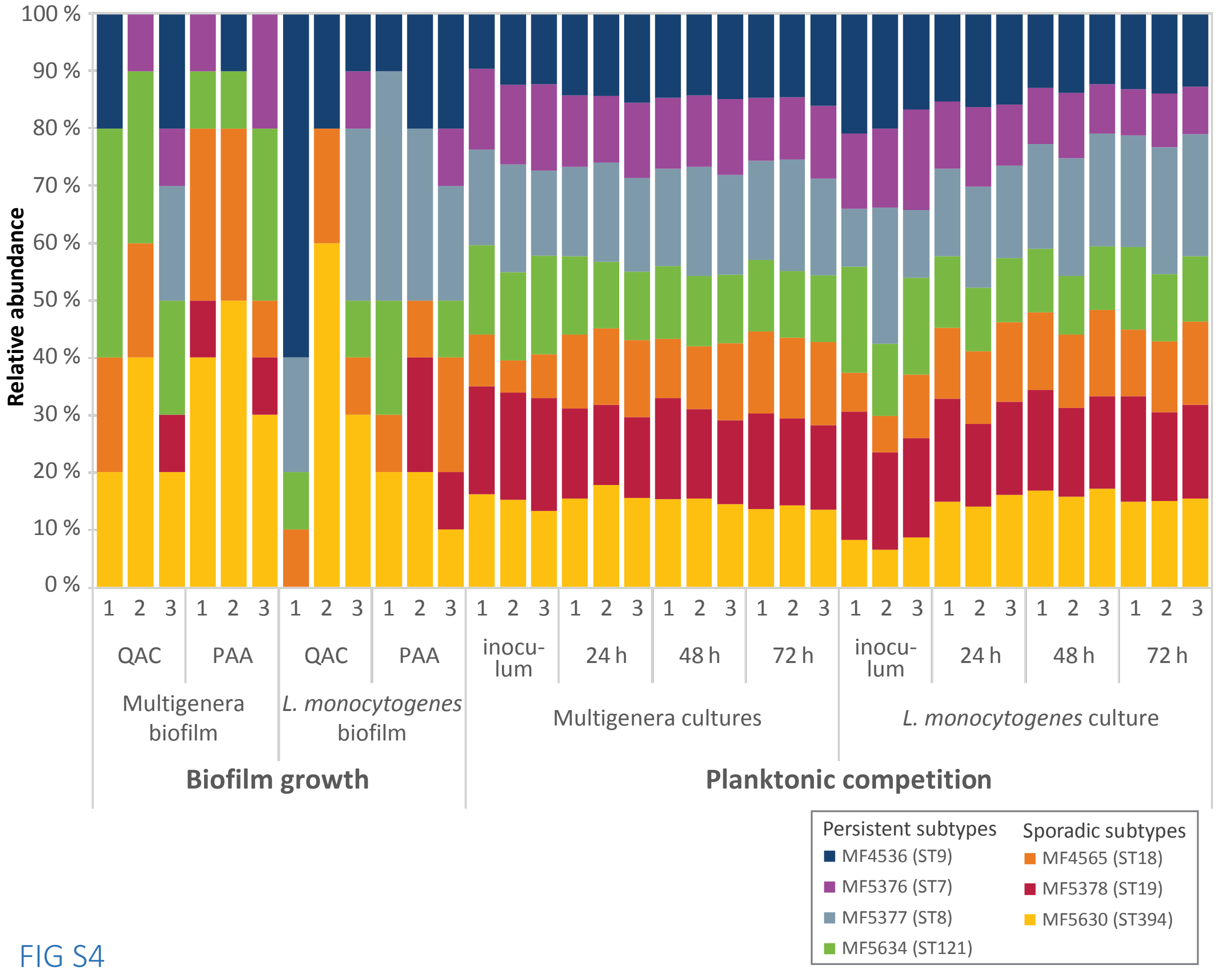

FIG S4

Competition between *L. monocytogenes* strains during biofilm and planktonic growth, showing results from individual experiments. Numbers 1 to 3 below each column refer to experiment number. Inoculated suspensions were composed of equal amounts of seven different *L. monocytogenes* strains, grown either together with 16 background microbiota strains (multigenera biofilm/culture) or alone (*L. monocytogenes* biofilm/culture). Results for biofilm growth shows frequencies of *L. monocytogenes* strains surviving in biofilms on conveyor belt coupons. Biofilms were allowed to develop for four days and then subjected to daily cleaning with Alkalifoam and disinfection with either a QAC-based or a PAA-based disinfection agent on four consecutive days before harvest. The identity of single colonies of *L. monocytogenes* was determined by Sanger sequencing of the *dapE* allele. Results in each column correspond to results for ten single *L. monocytogenes* colonies identified in one experiment. Planktonic competition cultures show the relative abundance of *L. monocytogenes* strains in time-course experiments, where cultures were grown with shaking in Erlenmeyer flasks for a total of 72 h. The frequencies of different *L. monocytogenes* strains was determined by *dapE* amplicon sequencing.

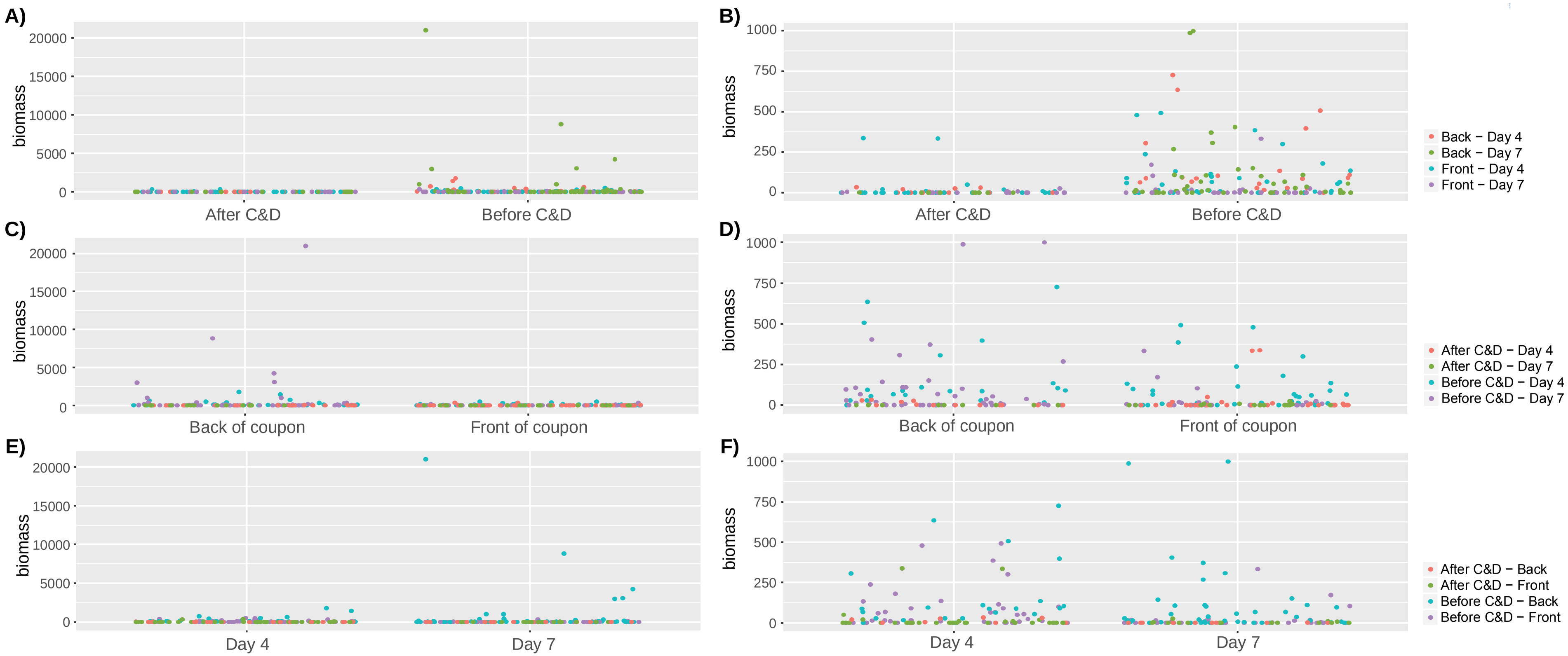

FIG S5

The biovolume of *L. monocytogenes* on conveyor belt coupons. The data is presented as beeswarm plots showing each individual measurement of biomass obtained from CLSM Z-scans. A) and B) shows the data grouped by samples collected before and after C&D; C) and D) compares the biomass measurements obtained on the front versus the back of coupons, while E) and F) compares samples collected after 4 and 7 days of biofilm growth. The same data is shown in A) and B), in C) and D), and in E) and F), respectively, except that the y-axis scale is different and only measurements of total volume of biomass ≤1000 μm^3 per scan are presented in B), D), and F). Different colored points represent different samples as listed in the legends to the right of each pair of graphs.