

Identification and first characterization of DinJ-YafQ toxin-antitoxin systems in *Lactobacillus* species of biotechnological interest

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Supplementary Table S1. Subset of *Lactobacillus* strains used for BRIG software analysis

Species	Strain	Chromosome Entry
<i>L. casei</i>	12A	NZ_CP006690.1/CP006690.1
<i>L. casei</i>	ATCC 393	NZ_AP012544.1/AP012544.1
<i>L. casei</i>	BL23	NC_010999.1/FM177140.1
<i>L. casei</i>	CECT 9104	NZ_LS991421.1/LS991421.1
<i>L. casei</i>	LC5	NZ_CP017065.1/CP017065.1
<i>L. casei</i>	W56	NC_018641.1/HE970764.1
<i>L. paracasei</i>	8700:2	NC_022112.1/CP002391.1
<i>L. paracasei</i>	ATCC 334	NC_008526.1/CP000423.1
<i>L. paracasei</i>	BD-II	NC_017474.1/CP002618.1
<i>L. paracasei</i>	CAUH35	NZ_CP012187.1/CP012187.1
<i>L. paracasei</i>	EG9	NZ_CP029546.1/CP029546.1
<i>L. paracasei</i>	FAM18149	NZ_CP017261.1/CP017261.1
<i>L. paracasei</i>	HD1.7	NZ_CP025582.1/CP025582.1
<i>L. paracasei</i>	HDS-01	NZ_CP026097.1/CP026097.1
<i>L. paracasei</i>	IIA	NZ_CP014985.1/CP014985.1
<i>L. paracasei</i>	JCM 8130	NZ_AP012541.1/AP012541.1
<i>L. paracasei</i>	KL1	NZ_CP013921.1/CP013921.1
<i>L. paracasei</i>	L9	NZ_CP012148.1/CP012148.1
<i>L. paracasei</i>	LC2W	NC_017473.1/CP002616.1
<i>L. paracasei</i>	LC355	NZ_CP029536.1/CP029536.1
<i>L. paracasei</i>	LOCK919	NC_021721.1/CP005486.1
<i>L. paracasei</i>	Lpc10	NZ_CP029686.1/CP029686.1
<i>L. paracasei</i>	N1115	NZ_CP007122.1/CP007122.1
<i>L. paracasei</i>	TK1501	NZ_CP017716.1/CP017716.1
<i>L. paracasei</i>	TMW 1.1434	NZ_CP016355.1/CP016355.1
<i>L. paracasei</i>	ZFM54	CP032637.1
<i>L. paracasei</i>	Zhang	NC_014334.2/CP001084.2
<i>L. rhamnosus</i>	4B15	NZ_CP021426.1/CP021426.1
<i>L. rhamnosus</i>	ATCC 11443	NZ_CP022109.1/CP022109.1
<i>L. rhamnosus</i>	ATCC 53103	NC_017482.1/AP011548.1
<i>L. rhamnosus</i>	ATCC 8530	NC_017491.1/CP003094.1
<i>L. rhamnosus</i>	BFE5264	NZ_CP014201.1/CP014201.1
<i>L. rhamnosus</i>	BPL5	NZ_LT220504.1/LT220504.1
<i>L. rhamnosus</i>	DSM 14870	NZ_CP006804.1/CP006804.1
<i>L. rhamnosus</i>	GG	NZ_CP031290.1/CP031290.1
<i>L. rhamnosus</i>	GG (ATCC 53103)	NC_013198.1/FM179322.1
<i>L. rhamnosus</i>	Lc 705	NC_013199.1/FM179323.1
<i>L. rhamnosus</i>	LOCK900	NC_021723.1/CP005484.1
<i>L. rhamnosus</i>	LOCK908	NC_021725.1/CP005485.1
<i>L. rhamnosus</i>	LR5	NZ_CP017063.1/CP017063.1
<i>L. rhamnosus</i>	LRB	CP016823.1
<i>L. rhamnosus</i>	Pen	NZ_CP020464.1/CP020464.1
<i>L. rhamnosus</i>	SCT-10-10-60	NZ_CP019305.1/CP019305.1

Supplementary Table S2. *Lactobacillus* strains used in this work

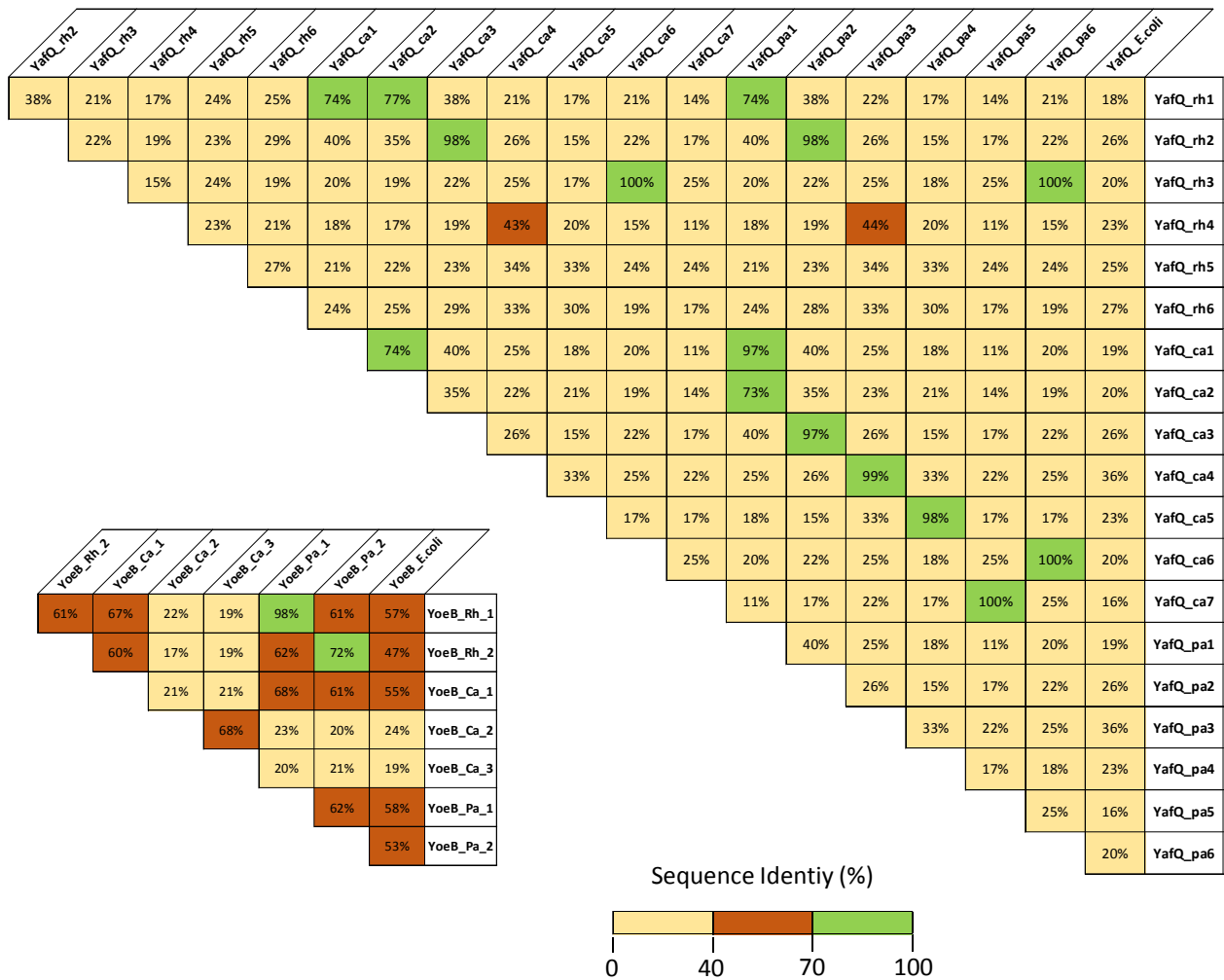
<i>Lactobacillus</i> species	Strain	Source
<i>L. rhamnosus</i>	1019, 1473, 2360	Parmigiano Reggiano cheese
<i>L. casei</i>	2046, 2094, 2057	Grana Padano cheese
<i>L. casei</i>	2306, 2240	Parmigiano Reggiano cheese
<i>L. casei</i>	2203, 4340	Milk
<i>L. paracasei</i>	2133, 2187	Grana Padano cheese
<i>L. paracasei</i>	4341, 4360, 4366, 4186	Milk
<i>L. paracasei</i>	2333	Parmigiano Reggiano cheese

Supplementary Table S3. *E. coli* strains and plasmids used in this work

<i>E. coli</i> strain	Description	Source
XL1-Blue	Cloning and plasmid purification, Tet ^R	Stratagene
C41(DE3) pLysS	Toxic activity assay, Cam ^R	Lucigen
Plasmid	Description	Source
pGEM-T easy	Cloning vector	Promega
pET11b	Expression vector, IPTG inducible, Amp ^R	Novagen
pET28b	Expression vector, IPTG inducible, Kan ^R	Novagen
pET11b-yafQ_pa4366	YafQ CDS from <i>L. paracasei</i> 4366 cloned in pET11b	This work
pET11b-yafQ_pa2333	YafQ CDS from <i>L. paracasei</i> 2333 cloned in pET11b	This work
pET28-dinJ_pa4366	DinJ CDS from <i>L. paracasei</i> 4366 cloned in pET28b	This work
pET11b-yafQ_rh2360	YafQ CDS from <i>L. rhamnosus</i> 2360 cloned in pET11b	This work
pET11b-yafQ_rh1473	YafQ CDS from <i>L. rhamnosus</i> 1473 cloned in pET11b	This work
pET28-dinJ_rh2360	DinJ CDS from <i>L. rhamnosus</i> 2360 cloned in pET28b	This work

Supplementary Table S4. Oligonucleotides used in this work

Primer	Sequence	Target strains
<i>dinJ-yafQ</i> identification		
dinj-yafQ_ca4_pa3 plus	AAGTGCTGCTCAAATCGTAGC	<i>L. casei</i> , <i>L. paracasei</i>
dinj-yafQ_ca4_pa3 minus	AAGGTTATGATGAGATCCGGTTC	<i>L. casei</i> , <i>L. paracasei</i>
dinj-yafQ_rh6 plus	CCATGTTCTTAATAGCATGGG	<i>L. rhamnosus</i>
dinj-yafQ_rh6 minus	TTACTCAATGTTCAATGTATCGCG	<i>L. rhamnosus</i>
YafQ and DinJ cloning		
yafQ_pa plus	CATATGTATAGTCTGGTTCCGACG	<i>L. paracasei</i> 4366, 2333
YafQ_pa minus	GGATCCTATTTACCCAGAAGGTTATGA	<i>L. paracasei</i> 4366, 2333
dinJ_pa4366 plus	CATATGGCAGCCACAAAGAAAGAA	<i>L. paracasei</i> 4366
dinJ_pa4366 minus	GGATCCCTATACATTCAAGTCTCTCCAC	<i>L. paracasei</i> 4366
yafQ_rh2360 plus	CATATGTTGACGATTAATCGCACG	<i>L. rhamnosus</i> 2360
yafQ_rh1473 plus	CATATGCAGCGTCAAGGTCATGTA	<i>L. rhamnosus</i> 1473
yafQ_rh minus	GGATCCTTACTCAATGTTCAATGTATCGC	<i>L. rhamnosus</i> 2360, 1473
dinJ_rh2360 minus	GGATCCTTAATCGTCAACATCATTGTATAA	<i>L. rhamnosus</i> 2360
dinJ_rh2360 plus	CATATGGAAACAAAATCCCGTATCA	<i>L. rhamnosus</i> 2360



Supplementary Figure S1. Sequence identities of YafQ and YoeB toxins identified in *Lactobacillus* genus. a) Percentages of identity among YafQ aminoacid sequences retrieved from PSI-BLAST and compared with *E. coli* YafQ (UniProt Q47149). b) Percentages of identity among YoeB aminoacid sequences retrieved from PSI-BLAST and compared with *E. coli* YoeB (UniProt P69348).

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dinJ-yafQ_ca2306 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_ca4340 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_ca2203 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_pa4186 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_pa2333 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_pa4366 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_pa4341 : ATGGCAGCC*CAAGAGAAAGAAATCCCTTGAATATTCGTTGAT-CGTGAAT*AAAAAGTCTGCTCAAATC*TAGCAAAATGATATGGGCATCC : 94
dinJ-yafQ_rh2360 : -----ATGGCAACAATAATCCCTATCCCGCTGAGATTGAAATCAACCAAAAGCAAGCA-GCTCTCCATGTTCTTAATCGCTGGGACTAG : 85
dinJ-yafQ_rh1473 : -----ATGGCAACAATAATCCCTATCCCGCTGAGATTGAAATCAACCAAAAGCAAGCA-GCTCTCCATGTTCTTAATCGCTGGGACTAG : 85
dinJ-yafQ_rh1019 : -----ATGCCAAAATAATCCCTATCCCGCTGAGATTGAAATCAACCAAAAGCAAGCA-GCTCTCCATGTTCTTAATCGCTGGGACTAG : 85

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dinJ-yafQ_pa2333 : 100 ACTTGAACCGCAGCTGTTACCGATGTTATGACGAAAATGGTGAAGAAGCAGGCCCTCCGTTTACCCAAAGTCT*CCAGTTGAACCTTACAG : 189
dinJ-yafQ_pa4366 : 100 ACTTGAACCGCAGCTGTTACCGATGTTATGACGAAAATGGTGAAGAAGCAGGCCCTCCGTTTACCCAAAGTCT*CCAGTTGAACCTTACAG : 189
dinJ-yafQ_pa4341 : 100 ACTTGAACCGCAGCTGTTACCGATGTTATGACGAAAATGGTGAAGAAGCAGGCCCTCCGTTTACCCAAAGTCT*CCAGTTGAACCTTACAG : 189
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dinJ-yafQ_rh1473 : 100 AGAGATTCTGGCTATTAACATGATTTGAAACGCATTTGGTGACACGGTGGTGGTGGATTTTACACTGAAATGTCGTGCTGATCAGCTTCA : 180
dinJ-yafQ_rh1019 : 100 ATATGTCCTCAGCTATTAACATGATTTGAAACGCATTTGGTGACACGGTGGTGGTGGATTTTACACTGAAATGTCGTGCTGATCAGCTTCA : 180

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dinJ-yafQ_rh1473 : 200 TTGTCAGAAAGCTGATGTTAAGGGGCGAATTAAGAGCTTCAAGACCGTGTGGTGCCTGATGAAAGATTT-----ATACAAATGATGTTGACCA : 269
dinJ-yafQ_rh1019 : 200 GTTGCAGAAAGCTGATGTTAAGGGGCGAATTAAGAGCTTCAAGACCGTGTGGTGCCTGATGAAAGATTT-----ATACAGTCATGTTGACCA : 269

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dinJ-yafQ_pa4186 : 300 CTTCACATTAAGCCCGATCTAAAACGATCTCCRAAGAGGATTGGCCATGGACGAACTAAAGACGGCTGTAAATCTCCTAGCCCTGGTACAAA : 378
dinJ-yafQ_pa2333 : 300 CTTCACATTAAGCCCGATCTAAAACGATCTCCRAAGAGGATTGGCCATGGACGAACTAAAGACGGCTGTAAATCTCCTAGCCCTGGTACAAA : 378
dinJ-yafQ_pa4366 : 300 CTTCACATTAAGCCCGATCTAAAACGATCTCCRAAGAGGATTGGCCATGGACGAACTAAAGACGGCTGTAAATCTCCTAGCCCTGGTACAAA : 378
dinJ-yafQ_pa4341 : 300 CTTCACATTAAGCCCGATCTAAAACGATCTCCRAAGAGGATTGGCCATGGACGAACTAAAGACGGCTGTAAATCTCCTAGCCCTGGTACAAA : 378
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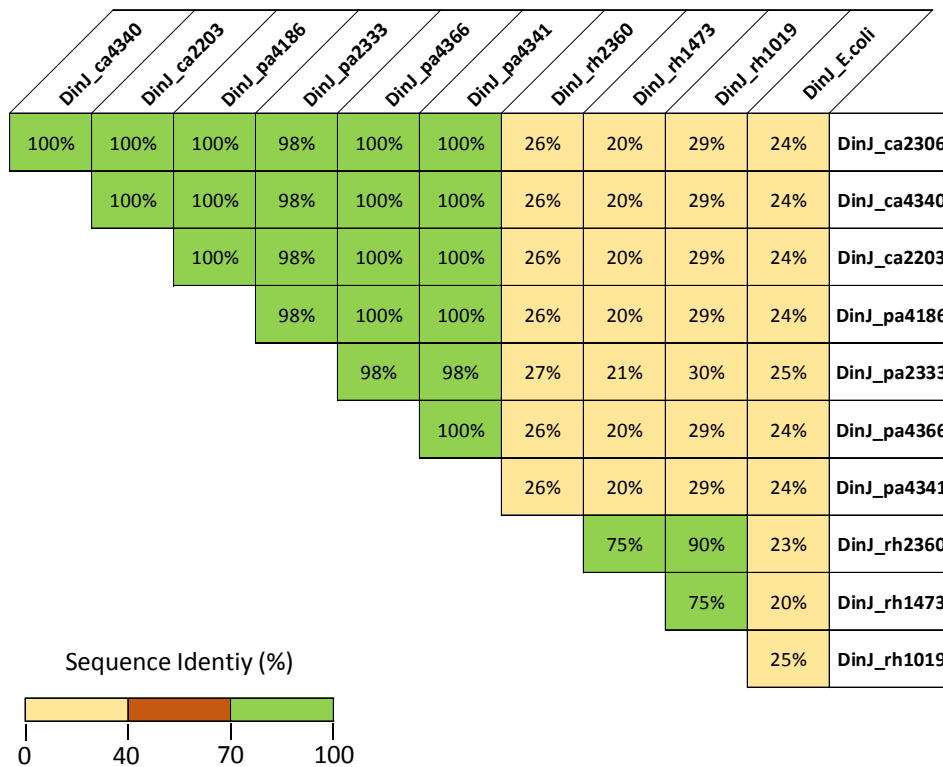
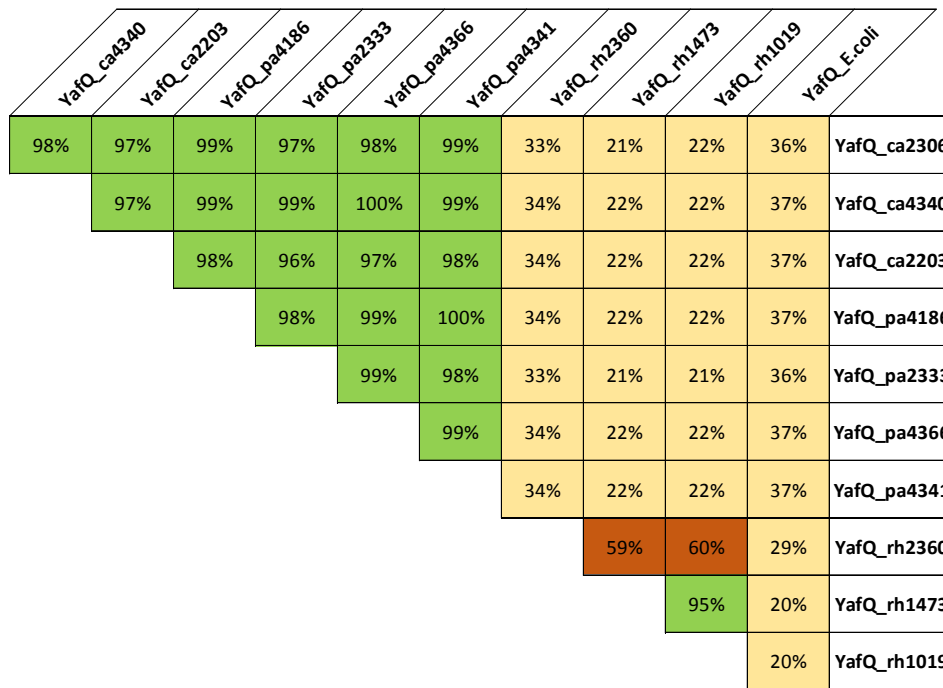
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dinJ-yafQ_pa4186 : 400 TECTGAACATTAAGCAAAAAGTATGCAGATCAAGCCCTTGTCTCAAGCAGCCGAGTGGAAAAGGATATCGTGAACATATTTTGACGGCCCTCGTG : 473
dinJ-yafQ_pa2333 : 400 TECTGAACATTAAGCAAAAAGTATGCAGATCAAGCCCTTGTCTCAAGCAGCCGAGTGGAAAAGGATATCGTGAACATATTTTGACGGCCCTCGTG : 473
dinJ-yafQ_pa4366 : 400 TECTGAACATTAAGCAAAAAGTATGCAGATCAAGCCCTTGTCTCAAGCAGCCGAGTGGAAAAGGATATCGTGAACATATTTTGACGGCCCTCGTG : 473
dinJ-yafQ_pa4341 : 400 TECTGAACATTAAGCAAAAAGTATGCAGATCAAGCCCTTGTCTCAAGCAGCCGAGTGGAAAAGGATATCGTGAACATATTTTGACGGCCCTCGTG : 473
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dinJ-yafQ_rh1473 : 400 CFTCAGATCGTGTCAAAATAGCTTCTTTA-CAGGACATGCTTTAAAGGCTGCTCAGAGCGGCGAAGCGAATTCGCTATGCT-----CTCGTG : 394
dinJ-yafQ_rh1019 : 400 CFTCAGATCGTGTCAAAATAGCTTCTTTA-CAGGACATGCTTTAAAGGCTGCTCAGAGCGGCGAAGCGAATTCGCTATGCT-----CTCGTG : 394

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dinJ-yafQ_pa4186 : 480 GCGACGGGTGCTAAATCTATAAAAATTAGCAGCAGAGATCTTATTTTAAACCTAGTTAGAAGCGGATCTCAGTCATAAAGCTTCTGGGTAATAG--- : 565
dinJ-yafQ_pa2333 : 480 GCGACGGGTGCTAAATCTATAAAAATTAGCAGCAGAGATCTTATTTTAAACCTAGTTAGAAGCGGATCTCAGTCATAAAGCTTCTGGGTAATAG--- : 565
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Operon	GenBank ID
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dinJ-yafQ_ca2203	MK544941
dinJ-yafQ_pa4186	MK544942
dinJ-yafQ_pa2333	MK544943
dinJ-yafQ_pa4366	MK544944
dinJ-yafQ_pa4341	MK544945
dinJ-yafQ_rh2360	MK544946
dinJ-yafQ_rh1473	MK544947
dinJ-yafQ_rh1019	MK544948

Supplementary Figure S2. Nucleotide sequence alignment and GenBank ID of the identified *dinJ-yafQ* operons shown in Figure 2a.



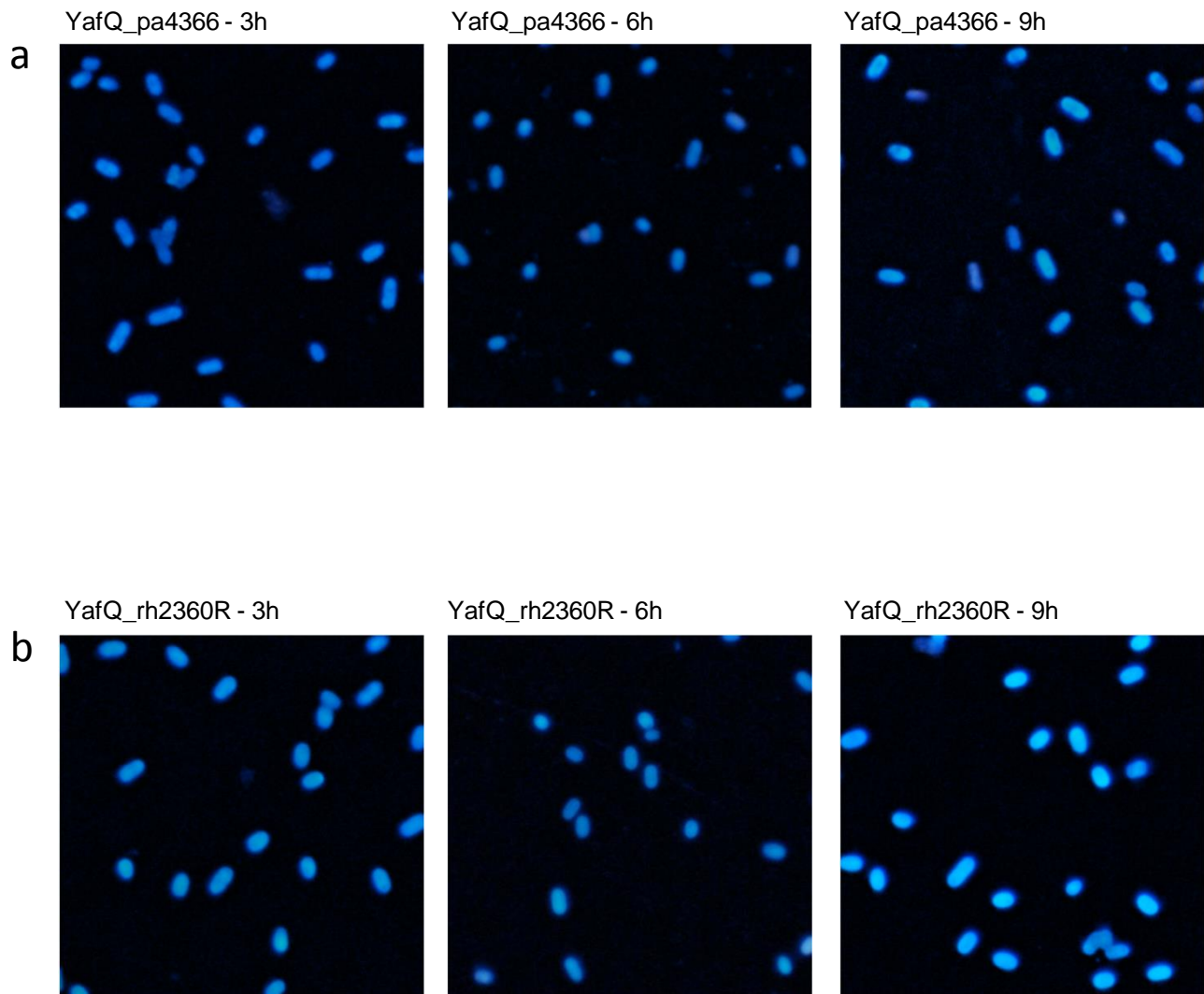
Supplementary Figure S3. Sequence identities of YafQ and DinJ proteins found in *L. casei*, *L. paracasei* and *L. rhamnosus*. a) Percentages of identity among the identified YafQ aminoacid sequences and with *E. coli* YafQ (UniProt Q47149). b) Percentages of identity among the identified DinJ aminoacid sequences and with *E. coli* DinJ (UniProt Q47150).

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      *      20      *      40      *      60      *      80      *      100
CP017261.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
CP012187.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
AP018392.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
CP029536.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
CP026097.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
CP025582.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
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CP013921.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
CP007122.1 : MYSLVPTPTFKRDLKRLSKKHWPMDLKTAVNLLAAGTNAELLSKKYADHALSSSEWKGyreLEVDGPRGdWLLIYKIECCDLILTLVRTGSHHNLLGK* : 100
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Supplementary Figure S4. Sequence alignment of YafQ_pa3 of *L. paracasei* strains retrieved from the databank.



Supplementary Figure S5. Membrane integrity assay. Fluorescence microscopy of *E. coli* C41(DE3) pLysS cells expressing *Lactobacillus* YafQ and stained with DAPI/EtBr dyes. (a) Cells producing YafQ_pa4366 toxin grown in LB/IPTG medium for 3, 6 or 9 hours. (b) Cells producing YafQ_rh2360R toxin grown in LB/IPTG medium for 3, 6 or 9 hours. Absence of the red EtBr fluorescence indicates membrane integrity.