Supplementary material

Development of *oriC*-based plasmids for *Mesoplasma florum*

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Running title: *Mesoplasma florum oriC* plasmids

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Supplementary Materials and Methods

Plasmids construction

(i) Molecular biology methods

Genes conferring resistance to tetracycline (*tetM)*, puromycin (*pac*), streptomycin and spectinomycin (*aadA1*), chloramphenicol (*cat*), and erythromycin (*ereB*) were recoded using a compromise codon table (Table S2) to obtain functional proteins (GenBank accession numbers: WP_000691749, AHL28657, WP_001206315, KLX70575, and WP_032488343, respectively) in *Escherichia coli* as well as in *Mesoplasma florum*. The *pac*, *aadA1*, and *cat* resistance genes were synthesized in gBlocks fragments (Integrated DNA Technologies), *ereB* resistance gene was obtained from Biobasic's gene synthesis service (*ereB*-pUC57), and *tetM* resistance cassette was amplified from the pTT01 plasmid (Table 3). All PCRs were performed using VeraSeq 2.0 DNA polymerase (Enzymatics) and primers listed in Table S1. PCR conditions were as follows: (i) 30 sec at 95°C; (ii) 30 cycles of 10 sec at 95°C, 30 sec at the appropriate annealing temperature, and 30 sec/kb at 72°C; (iii) 2 min at 72°C. PCR products were purified using Solid Phase Reversible Immobilization (SPRI) bead capture using Agencourt AMPure XP magnetic beads (Beckman Coulter) (1). Wild-type *M. florum* L1 genomic DNA (gDNA) was extracted using the Quick-gDNA MiniPrep kit (Zymo Research) according to the manufacturer's specifications. All plasmids generated in this study were cloned in chemically competent *E. coli* strain EC100D *pir*+ cells, except for pMflPT-o4 was cloned in *E. coli* strain MM294. Plasmid DNA was extracted using the EZ-10 Spin Column Plasmid DNA Minipreps kit (Biobasic) according to the manufacturer's instructions. Plasmid constructions were analyzed by restriction enzymes digestion, and *M. florum oriC* plasmids sequence was confirmed by paired-end Illumina

sequencing at the Laboratoire de Génomique Fonctionnelle de l'Université de Sherbrooke (QC, Canada). Plasmids sequence and annotations are available at http://lab-rodrigue.recherche.usherbrooke.ca/m_florum_plasmids/.

(ii) Construction of *M. florum oriC* plasmids

Plasmids and oligonucleotides used in this study are listed in Table 3 and in Table S1, respectively. *M. florum oriC* plasmids were constructed as depicted in Figure 2. The different *M. florum oriC* fragments were all amplified from *M. florum* L1 gDNA. Regions upstream and downstream of *dnaA*, designated *oriC1* and *oriC2* respectively, were amplified using *oriC1*-F/*oriC1*-R and *oriC2*-F/*oriC2*-R primer pairs. To build the *oriC3* fragment, *oriC1* and *oriC2* regions containing an overlapping sequence of 40 bp were amplified using *oriC1*-F/*oriC3*-R and *oriC3*-F/*oriC2*-R primer pairs. The *oriC3* fragment was then assembled by fusion PCR using flanking primers *oriC1*-F/*oriC2*-R. *dnaA* gene along with both upstream and downstream regions, designated *oriC4*, was first amplified in four distinct fragments using *oriC1*-F/*oriC4*-1-R, *oriC4*-1-F/*oriC4*-2-R, *oriC4*-2-F/*oriC4*-3-R and *oriC4*-3-F/*oriC2*-R primer pairs. The complete *oriC4* region was next assembled by fusion PCR using flanking primers *oriC1*-F/*oriC2*-R. *tetM* resistance cassette containing the pBOT1 promoter from pBOT1 plasmid (2) was amplified from pTT01 using *tetM*-F/*tetM*-R and *tetM*-1-F/*tetM*-R primer pairs to build pMflT-o1 and pMflT-o3/-o4, respectively. ColE1 replication origin was amplified from pUC19 (GenBank accession number: L09137) using colE1-F/colE1-R primers and $oriT_{RP4}$ was amplified from pSW23T (Genbank accession number: AY733066) using RP4-F/RP4-R primers. The colE1 and $oriT_{RP4}$ fragments were then assembled by fusion PCR using colE1-F/RP4-R flanking primers. PCR fragments *oriC1*, *oriC3,* and *oriC4* were assembled with the appropriate *tetM*, colE1, and

oriT$_{RP4}$ fragments using the Gibson Assembly Master Mix (New England BioLabs) to build pMflT-o1, pMflT-o3, and pMflT-o4, respectively. To generate pMflT-o2, pMflT-o4 was digested with ClaI, the resulting 3.6 kb band was purified using the Zymoclean Gel DNA Recovery kit (Zymo Research), and the purified fragment was circularized using the T4 DNA ligase.

(iii) Construction of *M. florum oriC* plasmids derivatives

In order to build pMflPT-o4, pMflST-o4, and pMflCT-o4 plasmids (see Fig. 2 and Table 3), pMflT-o4 was linearized using NotI and the corresponding resistance cassette was cloned using the Gibson Assembly Master Mix (New England BioLabs). For pMflPT-o4, pBOT1 promoter was amplified from pTT01 and *pac* resistance gene was amplified from gBlocks fragments using pBOT1-F/pBOT1-R and *pac*-F/*pac*-R primer pairs (Table S1), respectively. To construct the *pac* resistance cassette, pBOT1 and *pac* fragments were assembled by fusion PCR using pBOT1-F/*pac*-R flanking primers. For pMflST-o4, *aadA1* resistance cassette containing P_{N25} promoter (3) was amplified from gBlocks fragment using *aadA1-F*/*aadA1*-R primers. For pMflCT-o4, P_{N25} promoter and *cat* resistance gene were amplified from gBlocks fragments using P_{N25}-F/P_{N25}-R and *cat*-F/*cat*-R primer pairs, respectively. The P_{N25} and *cat* fragments were assembled by fusion PCR using P_{N25}-*F/cat*-R flanking primers to construct the *cat* resistance cassette. pMflET-o4 was constructed by cloning the *ereB* resistance cassette, obtained from *ereB*-pUC57 AscI/XhoI digestion, into a pMflT-o4 derivative plasmid.

(iiii) Construction of heterologous *oriC* plasmids

M. florum heterologous *oriC* plasmids were constructed as depicted in Figure S4B. pMflT-o4 backbone was amplified using pMfl-F/pMfl-R primer pair (Table S1), digested using

XhoI/PvuI, and dephosphorylated with Antarctic phosphatase (New England BioLabs). Heterologous *dnaA*/*oriC* regions were amplified from pMCO3 (*Mycoplasma capricolum subsp. capricolum*), pMYSO1 (*Mycoplasma mycoides subsp. mycoides*), pMYCO1 (*M. mycoides subsp. capri*), and pSD4 (*Spiroplasma citri*) plasmids (Table 3 and Fig. S4A) using *Mcap*-F/*Mcap*-R, *Mm*-F/*Mmm*-R, *Mm*-F/*Mmc*-R, and *Sci*-F/*Sci*-R primers pairs (Table S1), respectively. *OriC* fragments were then digested with XhoI/PvuI and phosphorylated using T4 phosphonucleotide kinase (Enzymatics). To construct pMcapT, pMmmT, pMmcT, and pSciT-o4 (Table 3 and Fig. S4B), pMflT-o4 backbone and the corresponding heterologous *oriC* fragment were ligated using T7 DNA ligase (New England BioLabs).

Supplementary Figures

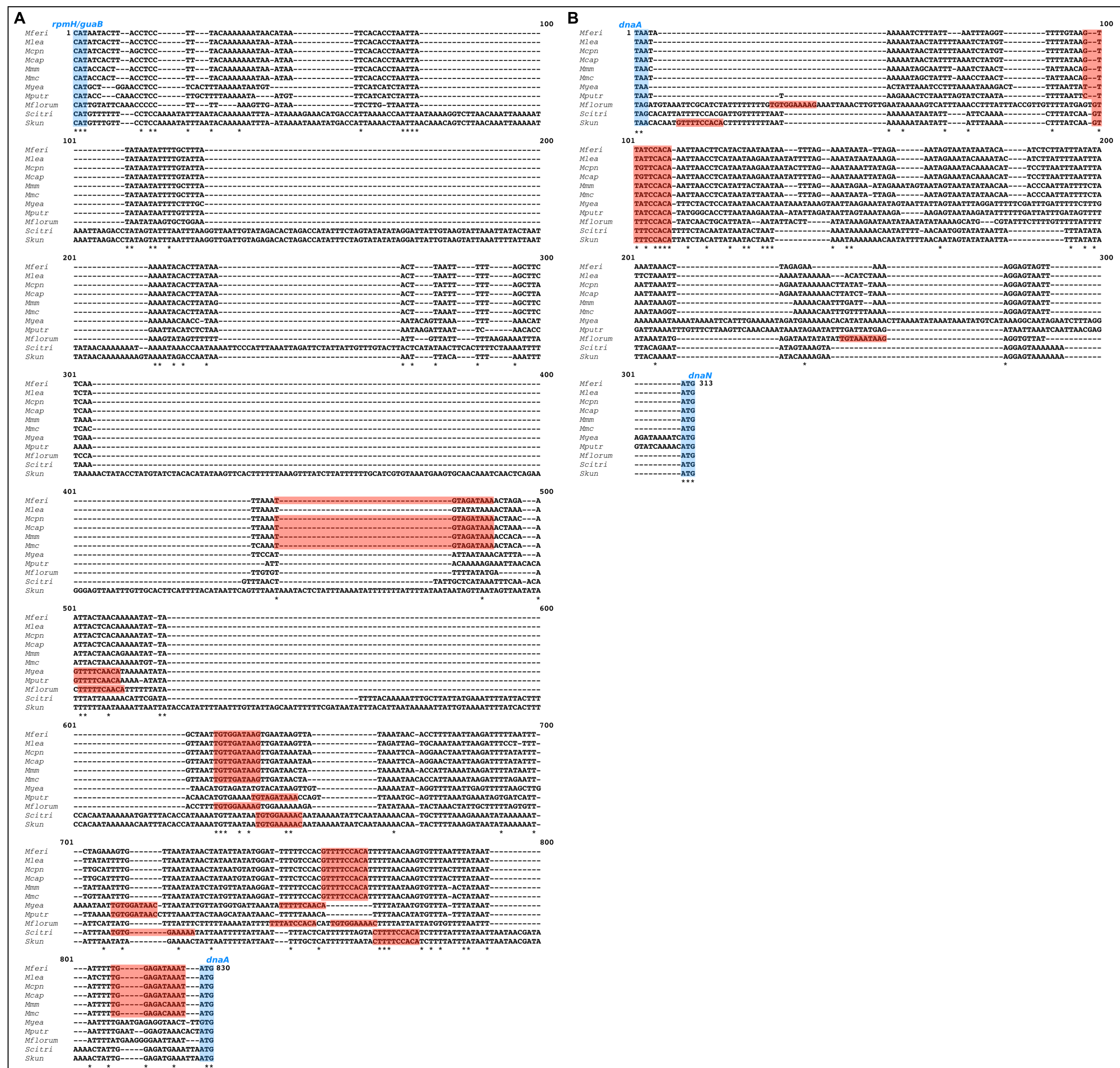

Fig. S1. DNA sequence alignment of the intergenic regions upstream (A) and downstream (B) of *dnaA* in selected species of the Spiroplasma group. Start and stop codons of surrounding genes are highlighted in blue, and putative DnaA boxes (see Fig. 1C) are highlighted in red. Perfectly conserved nucleotides are indicated with asterisks. *Mferi*, *Mycoplasma feriruminatoris*; *Mlea*,

Mycoplasma leachii; *Mcpn*, *M. capricolum subsp. capripneumoniae*; *Mcap*, *M. capricolum subsp. capricolum*; *Mmm*, *M. mycoides subsp. mycoides*; *Mmc*, *M. mycoides subsp. capri*; *Myea*, *Mycoplasma yeatsii*; *Mputr*, *Mycoplasma putrefaciens*; *Mflorum*, *M. florum*; *Scitri*, *S. citri*; *Skun*, *Spiroplasma kunkelii.*

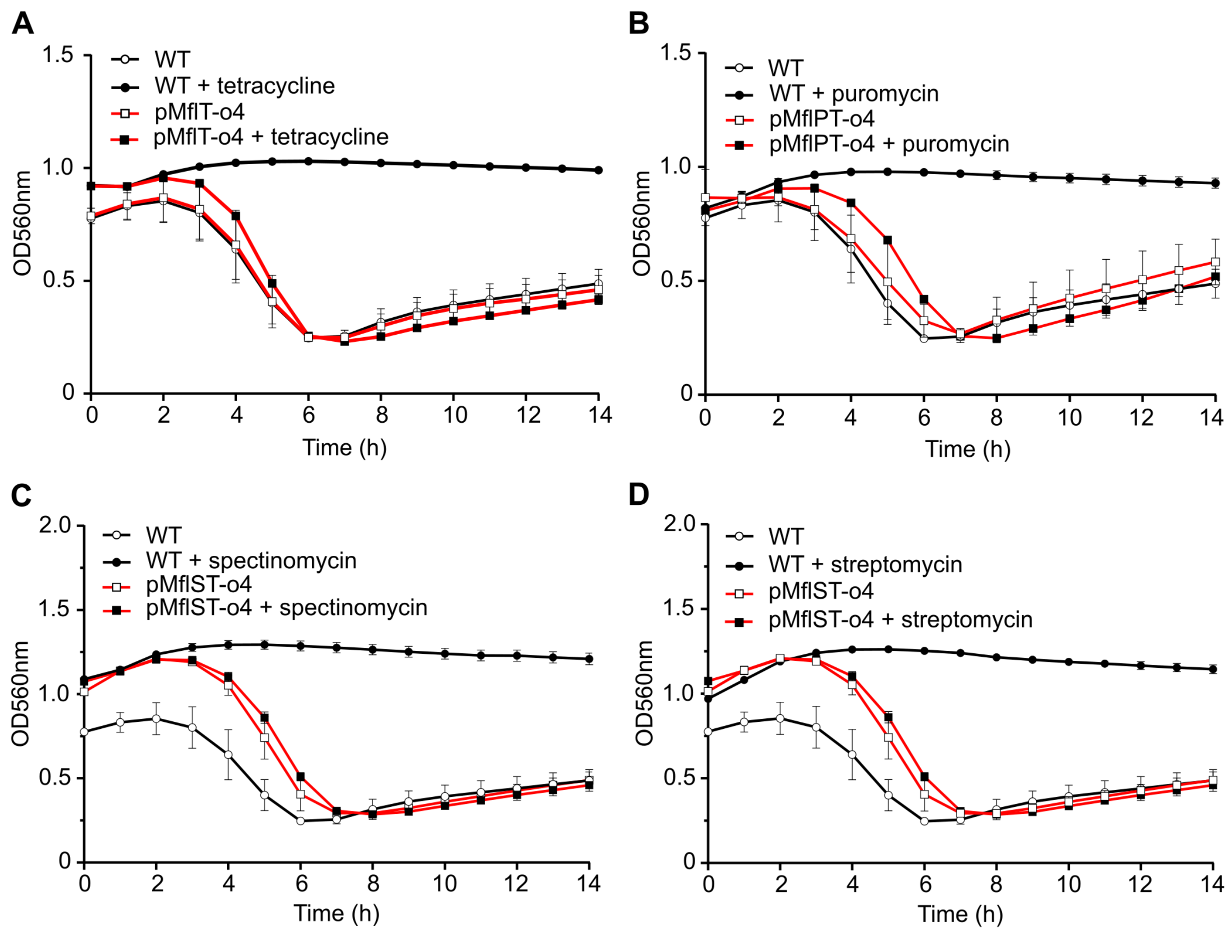

Fig. S2. Growth curves of *M. florum* L1 wild-type strain (WT) and *M. florum* L1 carrying pMflT-o4 (A), pMflPT-o4 (B) or pMflST-o4 (C and D) in ATCC 1161 medium with or without the indicated antibiotics. Growth was monitored by a decrease in absorbance of phenol red at 560 nm caused by the acidification of the culture medium that correlates with *M. florum* cell

concentrations (4). Antibiotics were used at the following concentrations: tetracycline, 15 µg/ml; puromycin, 30 µg/ml; spectinomycin and streptomycin, 100 µg/ml. Each data point represents the mean and the standard deviation of values obtained from three independent experiments.

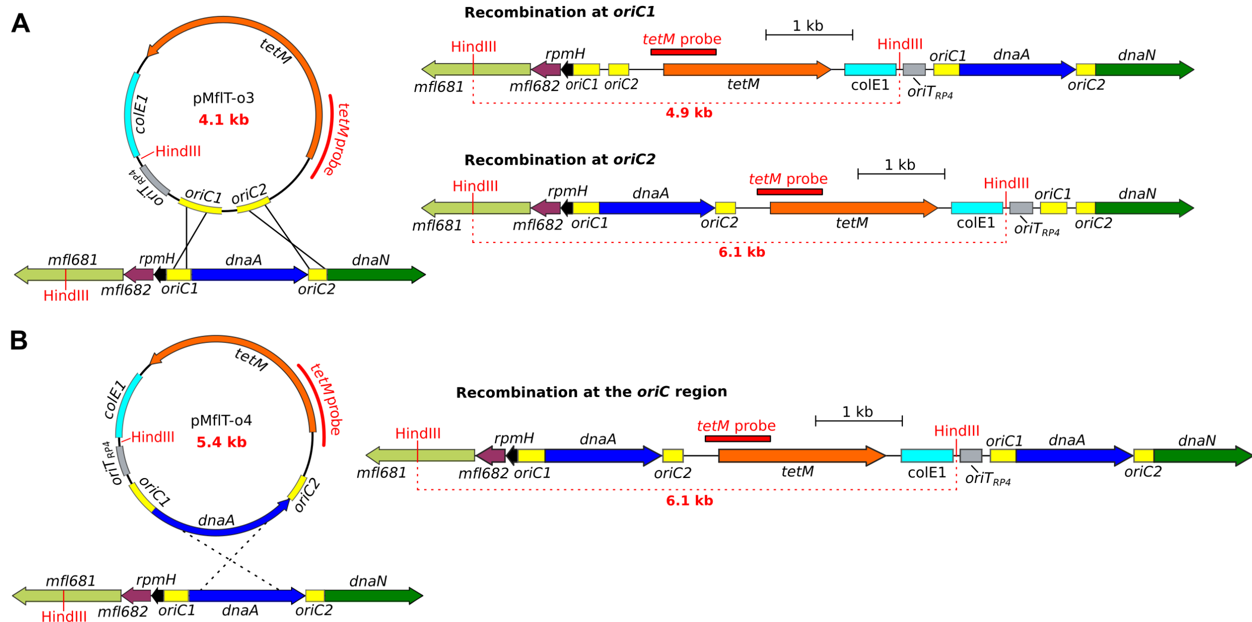

Fig. S3. Schematic representation of pMflT-o3 (A) and pMflT-o4 (B) plasmids recombination with the *oriC* region of the *M. florum* chromosome. The localization of HindIII sites and *tetM* probe used for Southern blot analysis are indicated in red, as well as the resulting fragment size. Genes are drawn to scale.

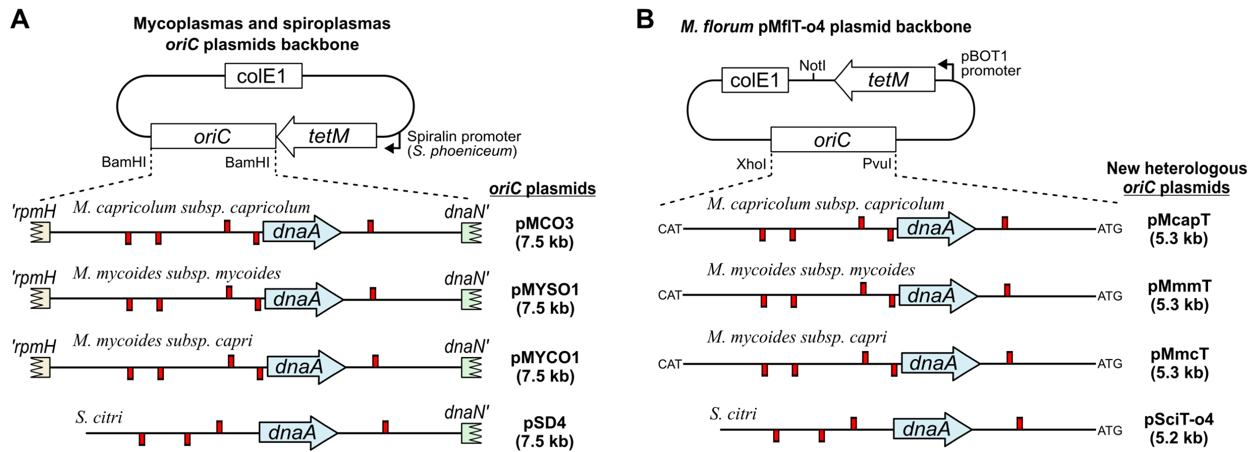

Fig. S4. Schematic representation of *M. florum* heterologous *oriC* plasmids. Putative DnaA boxes found within the intergenic regions upstream and downstream of *dnaA* are represented by red boxes as shown in Figure 1C. (A) Principal features of *M. capricolum*, *M. mycoides*, and *S. citri oriC* plasmids developed by Lartigue et al. and used in this study (2, 5, 6). (B) Depiction of the new *M. florum* heterologous *oriC* plasmids developed in this study (see Text S1 for construction details). Each plasmid contains a pMflT-o4 backbone in which the complete *M. florum oriC* region was replaced by a heterologous *oriC* region depicted in A.

Supplementary Tables

Table S1. Primers used in this study.

Name	**Nucleotide sequence (5' to 3')**
oriC1-F	GCGATGAGGATGAACCTACTCAGGGACCTGATGTGACGTGCCGTGAACACGAGCGTGTTT
oriC1-R	ATTAACCCTCACTAAAGGGAGTTTCCATATTAATTCCCCT
oriC2-F	GCGATGAGGATGAACCTACTCAGGGACCTGATGTGACGTGGTTTTAAGTGCTATTTCGCG
oriC2-R	ATTAACCCTCACTAAAGGGACTCATATAACACCTCTTATTTAC
oriC3-R	CGCGAAATAGCACTTAAAACGTTTCCATATTAATTCCCCT
oriC3-F	AGGGGAATTAATATGGAAACGTTTTAAGTGCTATTTCGCG

oriC4-1-R	CAATATATTCTTCAATAATGTCTTGGTCTATTAATTTTTCCTTCTTTAACTTA
oriC4-1-F	TAAGTTAAAGAAGGAAAAATTAATAGACCAAGACATTATTGAAGAATATATTG
oriC4-2-R	TAAAATAGTTACACCAAGATTACTTCTAACAAGGATTACGAACTCTG
oriC4-2-F	CAGAGTTCGTAATCCTTGTTAGAAGTAATCTTGGTGTAACTATTTTA
oriC4-3-R	CGCGAAATAGCACTTAAAACTGTACTATGGTCTCTTCCACCAAATTCTGCACC
oriC4-3-F	GGTGCAGAATTTGGTGGAAGAGACCATAGTACAGTTTTAAGTGCTATTTCGCG
tetM-F	AGGGGAATTAATATGGAAACTCCCTTTAGTGAGGGTTAAT
tetM-R	TATGCATCAGTATCGCATAACGACCATATAGCCCTACCTACTAAATTACCCTGTTATCCC
tetM-1-F	GTAAATAAGAGGTGTTATATGAGTCCCTTTAGTGAGGGTTAAT
colE1-F	TAGGTAGGGCTATATGGTCGTTATGCGATACTGATGCATAAAGGCCGCGTTGCTGGCGTT
colE1-R	GCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGTTGAGATCCTTTTTTTCTGC
RP4-F	CGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCGCTGCAGGAATTCGATATCA
RP4-R	CACGTCACATCAGGTCCCTGAGTAGGTTCATCCTCATCGCTGGGTACCAGCGCTTTTCCG
pBOT1-F	CGTTATATGTTCAACAAAATCACATAATAATACTAGTAGCTCCCTTTAGTGAGGGTTAAT
pBOT1-R	GTTGGTTTGTATTCAGTCATCTAGATTTCCTCCATTCAAA
pBOT2-F	GTCGTGACTGGGAAAACCCT
tetM-probe-R	TCCGTTTTGGTCAATTTTGTT
pac-F	TTTGAATGGAGGAAATCTAGATGACTGAATACAAACCAAC
pac-R	CCTAAATCGTATGCCCTATAGTGAGTCGTATTACTGCAGCTTATGCACCTGGTTTTCTTG
aadA1-F	CAAGAAAACCAGGTGCATAATCCCTTTAGTGAGGGTTAAT
aadA1-R	ATTAACCCTCACTAAAGGGATTATGCACCTGGTTTTCTTG
P_{N25}-*F*	CGTTATATGTTCAACAAAATCACATAATAATACTAGTAGCTCATAAAAAATTTATTTGCT
P_{N25}-*R*	CATCTAGATTTCCTCCATATAGT
cat-F	GATTCATACGACTCACTATATGGAGGAAATCTAGATGGAAAAAAAGATAACTGGGTACAC
cat-R	CCTAAATCGTATGCCCTATAGTGAGTCGTATTACTGCAGCTCGAGTTAAGCTCCACCTTG
qPCR-*tetM*-F	TGACCGTGCATATTCAGGTG
qPCR-*tetM*-R	TCACGTTGTTCAGGTTTGCT
qPCR-*rpoB*-F	CATGGCTGAAGCTGGAATGGAAAACTATGG
qPCR-*rpoB*-R	CGTTGTCCCCCGTTTTGTGC
qPCR-*rpoC*-F	CCTAAAGATGGAAAAGCGATTG
qPCR-*rpoC*-R	TCAACTGCAATCCCAATAACTG

pMfl-F	CTTATCTTAAAAGGAGTAATTATGCGATCGTCCCTTTAGTGAGGGTTAATGTCGTGACTG
pMfl-R	ATTTTTTTGTAAAGGAGGTAAGTGATATGCTCGAGCACGTCACATCAGGTCCCTGAGTAG
Mcap-F	CCTGATGTGACGTGCTCGAGCATATCACTTACCTCCTTTACAAAAAAATAAATAATTCAC
Mcap-R	ATTAACCCTCACTAAAGGGACGATCGCATAATTACTCCTTTTAAGATAAGTTTTTTATTC
Mm-F	TACTCAGGGACCTGATGTGACGTGCTCGAGCATACCACTACCTCCTTTACAAAAAAATAA
Mmm-R	CATTAACCCTCACTAAAGGGACGATCGCATAATTACTCCTTTTAATCAAATTGTTTTTAC
Mmc-R	CGACATTAACCCTCACTAAAGGGACGATCGCATAATTACTCCTTTTTTAAAACAAATTGT
Sci-F	TACTCAGGGACCTGATGTGACGTGCTCGAGTCGATATTTTACAAAAATTTGCTTATTATG
Sci-R	CATTAACCCTCACTAAAGGGACGATCGCATTTTTTTTACTCCTTACTTTAGTATATTCTG

Table S2. Compromise codon table for *M. florum* and *E. coli.*

Amino acid	Codon	Number in *M. florum*	Number / 1000 codons	*M. florum* Probability	*E. coli* Probability	Compromise probability[a]
Gly	GGG	848	3.45	0.06	.15	.12
Gly	GGA	5717	23.26	0.43	.11	.26
Gly	GGT	6147	25.01	0.47	.34	.49
Gly	GGC	461	1.88	0.03	.40	.13
Glu	GAG	1543	6.28	0.09	.31	.17
Glu	GAA	16303	66.34	0.91	.69	.83
Asp	GAT	10918	44.43	0.83	.63	.74
Asp	GAC	2203	8.96	0.17	.37	.26
Val	GTG	632	2.57	0.04	.37	.15
Val	GTA	4237	17.24	0.28	.15	.25
Val	GTT	9660	39.31	0.65	.26	.52
Val	GTC	344	1.40	0.02	.22	.08
Ala	GCG	519	2.11	0.04	.36	.15
Ala	GCA	6198	25.22	0.46	.21	.38
Ala	GCT	6297	25.62	0.47	.16	.34
Ala	GCC	522	2.12	0.04	.27	.13
Arg	AGG	194	0.79	0.03	.02	.05
Arg	AGA	5448	22.17	0.80	.04	.33
Ser	AGT	3752	15.27	0.23	.15	.23
Ser	AGC	935	3.80	0.06	.28	.16
Lys	AAG	2110	8.59	0.09	.23	.15
Lys	AAA	21721	88.38	0.91	.77	.85
Asn	AAT	13612	55.39	0.77	.45	.62
Asn	AAC	4161	16.93	0.23	.55	.38
Met	ATG	5660	23.03	1.00	1.00	1.00

Ile	ATA	7748	31.53	0.32	.07	.17
Ile	ATT	14701	59.82	0.60	.51	.62
Ile	ATC	1968	8.01	0.08	.42	.21
Thr	ACG	211	0.86	0.02	.27	.10
Thr	ACA	7243	29.47	0.55	.13	.38
Thr	ACT	5558	22.62	0.42	.17	.38
Thr	ACC	274	1.11	0.02	.44	.14
Trp	TGG	101	0.41	0.04	1.00	1.00
Trp	TGA	2489	10.13	0.96	0.0	0.0
Cys	TGT	1275	5.19	0.85	.45	.68
Cys	TGC	228	0.93	0.15	.55	.32
End	TAG	132	0.54	0.19	.07	.14
End	TAA	551	2.24	0.81	.64	.86
Tyr	TAT	7191	29.26	0.78	.57	.68
Tyr	TAC	1972	8.02	0.22	.43	.32
Leu	TTG	1765	7.18	0.08	.13	.16
Leu	TTA	15736	64.03	0.71	.13	.49
Phe	TTT	10293	41.88	0.81	.57	.70
Phe	TTC	2391	9.73	0.19	.43	.30
Ser	TCG	206	0.84	0.01	.16	.05
Ser	TCA	7413	30.16	0.46	.12	.29
Ser	TCT	3585	14.59	0.22	.15	.22
Ser	TCC	92	0.37	0.01	.15	.05
Arg	CGG	6	0.02	0.00	.10	0.0
Arg	CGA	128	0.52	0.02	.06	.06
Arg	CGT	998	4.06	0.15	.38	.44
Arg	CGC	67	0.27	0.01	.40	.12
Gln	CAG	404	1.64	0.05	.65	.24
Gln	CAA	7058	28.72	0.95	.35	.76
His	CAT	2211	9.00	0.73	.57	.65
His	CAC	836	3.40	0.27	.43	.35
Leu	CTG	182	0.74	0.01	.50	.11
Leu	CTA	1690	6.88	0.08	.04	.08
Leu	CTT	2682	10.91	0.12	.10	.17
Leu	CTC	52	0.21	0.00	.10	0.0
Pro	CCG	203	0.83	0.03	.52	.16
Pro	CCA	3516	14.31	0.57	.19	.44
Pro	CCT	2306	9.38	0.37	.16	.32
Pro	CCC	157	0.64	0.03	.12	.08

[a]The compromise probability is calculated by taking the geometric mean of the two genome codon probabilities and renormalizing such that the sum of probabilities of each codon for a specific amino acid is unity.

Table S3. *E. coli* and *M. florum* mating ratios for pMflT-o4 conjugation.

E. coli MFD*pir*			*M. florum* L1			Mating volume ratio (*M. florum* / *E. coli*)
Volume[a] (ml)	Dilution	Approx. CFU	Volume[b] (ml)	Dilution	Approx. CFU	
1	undiluted	2.5×10^7	1	undiluted	5×10^9	10^0
1	undiluted	2.5×10^7	0.1	undiluted	5×10^8	10^{-1}
1	undiluted	2.5×10^7	0.01	undiluted	5×10^7	10^{-2}
1	undiluted	2.5×10^7	0.01	10^{-1}	5×10^6	10^{-3}
1	undiluted	2.5×10^7	0.01	10^{-2}	5×10^5	10^{-4}
1	undiluted	2.5×10^7	-	-	-	No recipient control
-	-	-	1	undiluted	5×10^9	No donor control
-	-	-	1	undiluted	5×10^9	Purified plasmid control (1 µg of pMflT-o4)

[a]Washed *E. coli* cells from a ~2.5×10^7 CFU/ml culture.
[b]Washed *M. florum* cells from a ~5×10^9 CFU/ml culture.

Table S4. *OriC* region percentage identity matrix of selected species of the Spiroplasma group.

Species	*Mferi*	*Mlea*	*Mcpn*	*Mcap*	*Mmm*	*Mmc*	*Myea*	*Mputr*	*Mflorum*	*Scitri*	*Skun*
Mferi	100%	90%	90%	90%	90%	90%	74%	73%	64%	62%	62%
Mlea		100%	94%	94%	91%	91%	73%	72%	63%	62%	62%
Mcpn			100%	98%	91%	92%	73%	72%	64%	62%	62%
Mcap				100%	92%	92%	73%	72%	63%	62%	63%
Mmm					100%	97%	72%	72%	62%	62%	62%
Mmc						100%	72%	73%	62%	62%	62%
Myea							100%	74%	61%	59%	60%
Mputr								100%	61%	59%	59%
Mflorum									100%	57%	57%
Scitri										100%	94%
Skun											100%

Mferi, *M. feriruminatoris*; *Mlea*, *M. leachii*; *Mcpn*, *M. capricolum subsp. capripneumoniae*; *Mcap*, *M. capricolum subsp. capricolum*; *Mmm*, *M. mycoides subsp. mycoides*; *Mmc*, *M. mycoides subsp. capri*; *Myea*, *M. yeatsii*; *Mputr*, *M. putrefaciens*; *Mflorum*, *M. florum*; *Scitri*, *S. citri*; *Skun*, *S. kunkelii.*

Table S5. Putative DnaA boxes found within the *oriC* intergenic regions of selected species of the Spiroplasma group.

DnaA box sequence	Strand	*p*-value	Genomic position (bp)	Position relative to *dnaA* start codon (bp)
M. feriruminatoris				
rpmH/dnaA				
TTTATCTACA	-	4.8E-5	4,332 – 4,341	(-182) – (-191)
CTTATCCACA	-	6.0E-6	4,374 – 4,383	(-140) – (-149)
GTTTTCCACA	+	2.2E-6	4,471 – 4,480	(-43) – (-52)
TTTATCTCCA	-	6.3E-5	4,512 – 4,521	(-2) – (-11)
dnaA/dnaN				
GTTATCCACA	+	1.1E-6	5,908 – 5,917	1,386 – 1,395
M. leachii				
rpmH/dnaA				
CTTATCAACA	-	3.4E-5	79 – 88	(-139) – (-148)
GTTTTCCACA	+	2.2E-6	175 – 184	(-43) – (-52)
TTTATCTCCA	-	6.3E-5	216 – 225	(-2) – (-11)
dnaA/dnaN				
GTTATTCACA	+	4.2E-5	1,614 – 1,623	1,388 – 1,397
M. capricolum subsp. capripneumoniae				
rpmH/dnaA				

TTTATCTACA	-	4.8E-5	1,017,103 – 1,017,112	(-182) – (-191)
CTTATCAACA	-	3.4E-5	1,017,145 – 1,017,154	(-140) – (-149)
GTTTTCCACA	+	2.2E-6	1,017,242 – 1,017,251	(-43) – (-52)
TTTATCTCCA	-	6.3E-5	1,017,283 – 1,017,292	(-2) – (-11)
dnaA/dnaN				
GTTGTTCACA	+	5.6E-5	1,388 – 1,397	1,388 – 1,397
M. capricolum subsp. capricolum				
rpmH/dnaA				
TTTATCTACA	-	4.8E-5	1,009,833 – 1,009,842	(-182) – (-191)
CTTATCAACA	-	3.4E-5	1,009,875 – 1,009,884	(-140) – (-149)
GTTTTCCACA	+	2.2E-6	1,009,972 – 1,009,981	(-43) – (-52)
TTTATCTCCA	-	6.3E-5	1,010,013 – 1,010,022	(-2) – (-11)
dnaA/dnaN				
GTTGTTCACA	+	5.6E-5	1,388 – 1,397	1,388 – 1,397
M. mycoides subsp. mycoides[a]				
rpmH/dnaA				
TTTATCTACA	-	4.8E-5	1,211,602 – 1,211,611	(-180) – (-189)
CTTATCAACA	-	3.4E-5	1,211,644 – 1,211,653	(-138) – (-147)
GTTTTCCACA	+	2.2E-6	37 – 46	(-42) – (-51)
TTTGTCTCCA	-	7.4E-5	77 – 86	(-2) – (-11)
dnaA/dnaN				
GTTATCCACA	+	1.1E-6	1,474 – 1,483	1,387 – 1,396
M. mycoides subsp. capri				
rpmH/dnaA				
TTTATCTACA	-	4.8E-5	1,078,118 – 1,078,127	(-181) – (-190)
CTTATCAACA	-	3.4E-5	1,078,160 – 1,078,169	(-139) – (-148)
GTTTTCCACA	+	2.2E-6	1,078,257 – 1,078,266	(-42) – (-51)
TTTGTCTCCA	-	7.4E-5	1,078,297 – 1,078,306	(-2) – (-11)
dnaA/dnaN				
GTTATCCACA	+	1.1E-6	1,387 – 1,396	1,387 – 1,396
M. yeatsii[b]				
rpmH/dnaA				
GTTTTCAACA	+	2.3E-5	894,886 – 894,895	(-172) – (-181)
GTTATCCACA	-	1.1E-6	894,975 – 894,984	(-83) – (-92)
TTTTTCAACA	+	5.6E-5	895,010 – 895,019	(-48) – (-57)
dnaA/dnaN				
TTTATCCACA	+	1.2E-5	1,425 – 1,434	1,410 – 1,419
M. putrefaciens				
rpmH/dnaA				
GTTTTCAACA	+	2.3E-5	832,426 – 832,435	(-169) – (-178)
TTTATCTACA	-	4.8E-5	832,459 – 832,468	(-136) – (-145)
GTTATCCACA	-	1.1E-6	832,513 – 832,522	(-82) – (-91)
dnaA/dnaN				
CTTATCCACA	+	6.0E-6	1,392 – 1,401	1,392 – 1,401
M. florum				
rpmH/dnaA				
TTTTTCAACA	+	5.6E-5	793,045 – 793,054	(-171) – (-180)
CTTTTCCACA	-	7.1E-6	793,070 – 793,079	(-146) – (-155)
TTTATCCACA	+	1.2E-5	793,157 – 793,166	(-59) – (-68)
GTTTTCCACA	-	2.2E-6	793,170 – 793,179	(-46) – (-55)

dnaA/dnaN				
CTTTTCCACA	-	7.1E-6	1,359 – 1,368	1,359 – 1,368
GTTTTCCACA	+	2.2E-6	1,428 – 1,437	1,428 – 1,437
CTTATTTACA	-	9.6E-5	1,541 – 1,550	1,541 – 1,550
S. citri				
guaB/dnaA				
GTTTTCCACA	-	2.2E-6	52,010 – 52,019	(-159) – (-168)
TTTTTCCACA	-	1.3E-5	52,075 – 52,084	(-94) – (-103)
CTTTTCCACA	+	7.1E-6	52,120 – 52,129	(-49) – (-58)
dnaA/dnaN				
GTTTTCCACA	+	2.2E-6	1,413 – 1,422	1,413 – 1,422
S. kunkelii				
guaB/dnaA				
GTTTTTCACA	-	4.5E-5	1,463,816 – 1,463,825	(-159) – (-168)
CTTTTCCACA	+	7.1E-6	1,463,926 – 9	(-49) – (-58)
dnaA/dnaN				
GTTTTCCACA	+	2.2E-6	1,417 – 1,426	1,360 – 1,369
GTTTTCCACA	+	2.2E-6	1,470 – 1,479	1,413 – 1,422

[a]According to multiple DNA sequence alignment, protein blast, and absence of putative Shine-Dalgarno sequence, the start codon of *dnaA* was considered to be located at position 88 bp instead of position 1 bp, and the start codon of *rpmH* was considered to be located at position 1,211,498 bp instead of 1,211,534 bp, relatively to the available Genbank sequence (RefSeq NC_005364.2).

[b]According to multiple DNA sequence alignment, protein blast, and absence of putative Shine-Dalgarno sequence, the start codon of *dnaA* was considered to be located at position 16 bp instead of position 1 bp, relatively to the available Genbank sequence (RefSeq NZ_CP007520.1).

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