

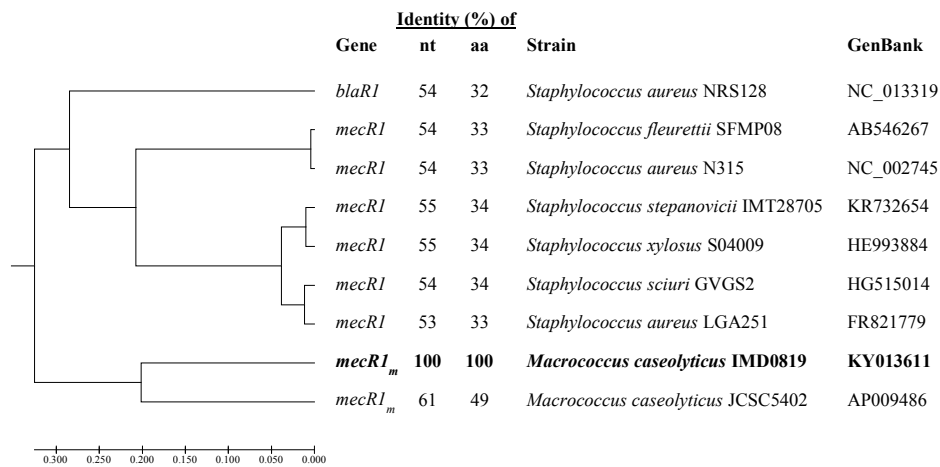
Novel methicillin resistance gene *mecD* in clinical *Micrococcus caseolyticus* strains from bovine and canine sources

Sybille Schwendener, Kerstin Cotting, Vincent Perreten*

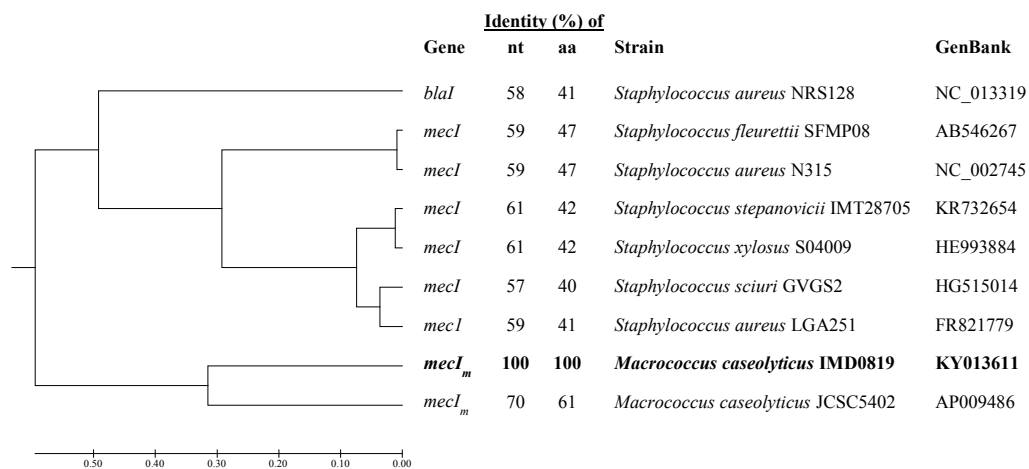
Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

*Corresponding author. Mailing address: Institute of Veterinary Bacteriology, University of Bern, Länggassstrasse 122, CH-3012 Bern, Switzerland, Phone: +41 31 631 24 30. Fax: +41 31 631 26 34. E-mail: vincent.perreten@vetsuisse.unibe.ch.

Supplementary Figures



Supplementary Figure S1. Phylogenetic tree of the receptors *mecR1/blaR1* of homologous systems consisting of *mec-mecR1-mecI* or *blaZ-blaR1-blaI* operon. Evolutionary analysis was performed for nucleotide sequences using the UPGMA method in MEGA7 [Mol. Biol. Evol. 2016; 33(7):1870-4.]. The percentage of nucleotide (nt) and amino acid (aa) identity between *mecR1_m* of *M. caseolyticus* strain IMD0819 and other *mecR1/blaR1* receptors was determined by sequence alignment with Clustral OMEGA [<http://www.ebi.ac.uk/Tools/msa/clustalo/>]. Note: the *mecR1* sequence of *S. saprophyticus* strain 210 is only partially available in the GenBank and was therefore omitted. The *mecA1* gene of *S. sciuri* and the *mecA2* gene of *S. vitulinus* are not preceded by regulator genes.



Supplementary Figure S2. Phylogenetic tree of the transcriptional repressors *mecl/blaI* of homologous systems consisting of *mec-mecRI-mecI* or *blaZ-blaRI-blaI* operon. Evolutionary analysis was performed for nucleotide sequences using the UPGMA method in MEGA7 [Mol. Biol. Evol. 2016; 33(7):1870-4.]. The percentage of nucleotide (nt) and amino acid (aa) identity between *mecl_m* of *M. caseolyticus* strain IMD0819 and other *mecl/blaI* repressors was determined by sequence alignment with Clustral OMEGA [http://www.ebi.ac.uk/Tools/msa/clustalo/]. Note: *mecl* sequence of *S. saprophyticus* strain 210 is not available in the GenBank, and the *mecAI* gene of *S. sciuri* and the *mecA2* gene of *S. vitulinus* are not preceded by regulator genes.

Supplementary Tables

Supplementary Table S1. Oligonucleotide primers.

Primer name	Primer label in Figure 3	Sequence (5'-3')	Target	Reference
mecA-1	-	AAAATCGATGGTAAAGGTTGGC	<i>mecA</i>	J. Clin. Microbiol. 2002; 40(8):2786-90.
mecA-2	-	AGTTCTGCAGTACCGGATTTGC	<i>mecA</i>	J. Clin. Microbiol. 2002; 40(8):2786-90.
mecB-fw	-	TGTTCCGGCATTTCGACGAA	<i>mecB</i>	Antimicrob. Agents Chemother. 2015; 59(8):4577-83.
mecB-rv	-	TCTCCCTGGCCATATCCTGA	<i>mecB</i>	Antimicrob. Agents Chemother. 2015; 59(8):4577-83.
mecC-F	-	CAGCCAGATTCATTGTACC	<i>mecC</i>	J. Dairy Sci. 2013; 96(4):2247-57.
mecC-R	-	AACATCGTACGATGGGGTAC	<i>mecC</i>	J. Dairy Sci. 2013; 96(4):2247-57.
mecD-F	a	TCCTTTAGCGATAGATGGTGAA	<i>mecD</i>	This study
mecD-R	b	CTCCCATCTTTTCTCCATCCT	<i>mecD</i>	This study
mecD-XhoI-F	-	TTATA <u>CTCGAG</u> ATTACACCTCCAATTCTATTATATCATG	<i>mecD</i> operon	This study
mec-XhoI-F	-	TTATA <u>CTCGAG</u> CTGACTTCTCACTTAGTTTGAG	<i>mecD</i> operon	This study
mecD-SpeI-R	-	TTATA <u>ACTAGT</u> TTATTCTGAGTCTGGTTCATAAGTC	<i>mecD</i> operon	This study
truA-F	c	GACAGTATCCCTGCAATCATTC	<i>truA</i>	This study
s66-R	d	ACCAACCCGCATACTTCATC	<i>s66</i>	This study
int-0819-F	e	TGGCTAAGGACAAAAGATCAG	<i>int</i> of McRI _{<i>mecD</i>-1}	This study
int-0473-F	f	TCATGGCTTCAGGCATACAC	<i>int</i> of McRI _{<i>mecD</i>-2}	This study
araC-F	g	TACCGTCATTCTGGCAAAC	<i>araC</i>	This study
orf21-R	h	GCTCCATTGCCCATAGAC	<i>orf21</i>	This study
orf20-F	i	GTATTCCCAACTTCGTCTGGA	<i>orf20</i>	This study
cop-R	j	GCAAGAATTAATACAATCCAATCTG	<i>cop</i>	This study

Restriction sites used for cloning are underlined.

Supplementary Table S2. PCR and restriction analysis for detection of McRI_{*mecD*-1} and McRI_{*mecD*-2} in *M. caseolyticus*.

Template	Forward primer (5' region)	Reverse primer (3' region)	Amplicon size/bp	Restriction enzyme	Sizes of restriction fragment/bp
<i>mecD</i> -positive					
IMD0819	truA-F	mecD-R	11,030	HindIII	606 + 1,855 + 3,854 + 4,715
	mecD-F	s66-R	19,771	HincII	994 + 1,621 + 2,551 + 2,756 + 4,871 + 6,978
KM0211	truA-F	mecD-R	11,030	HindIII	606 + 1,855 + 3,854 + 4,715
	mecD-F	s66-R	14,920	HincII	994 + 1,621 + 2,127 + 2,551 + 2,756 + 4,871
IMD0473	truA-F	mecD-R	10,846	HindIII	52 + 781 + 1,579 + 1,855 + 2,120 + 4,459
	mecD-F	s66-R	8,114	HincII	2,104 + 2,356 + 3,654
<i>mecD</i> -negative					
KM1352	truA-F	s66-R	6,950	EcoRI	607 + 1,346 + 1,584 + 3,413

Supplementary Table S3. References for integrases used to construct phylogenetic tree of Figure 4.

Strain	Protein-ID	GenBank/ Nucleotide position	Reference
<i>M. caseolyticus</i> IMD0819		KY013611/ 8427-7261	This study
<i>M. caseolyticus</i> KM0211		KY013612/ 8427-7261	This study
<i>M. caseolyticus</i> IMD0473		KY013610/ 8233-7076	This study
<i>M. caseolyticus</i> JCSC5402	WP_012656138.1	AP009484.1/ 223907-222741	J. Bacteriol. 2009; 191(4):1180-90.
<i>S. aureus</i> 930918-3	WP_000161346.1	ABFA01000015.1/ 9951-11117	BMC Genomics. 2008; 9:433.
<i>Lysinibacillus</i> sp. A1	KHK50488.1	JSZM01000021.1/ 109128-107962	Genome Announc. 2015; 3(2). pii: e00095-15.
<i>B. chagannorensis</i> DSM 18086	WP_026696990.1	AUCK01000006.1/ 56493-55324	Int. J. Syst. Evol. Microbiol. 2007; 57(Pt 9):2084-8.
<i>B. hemicellulosilyticus</i> JCM 9152	GAE31526.1	BAZO01000020.1/ 59332-58589	Genome Announc. 2014; 2(1):e01258-13.
<i>S. intermedius</i> NCTC 11048	WP_019168674.1	CAIB01000160.1/ 37188-36052	Front. Cell. Infect. Microbiol. 2012; 2:44.
<i>S. hominis</i> LRKNS031	OAW30112.1	LXRS01000041.1/ 43073-41937	Direct Submission: Maurer, Baesler and Knobloch, 2016.
<i>S. aureus</i> V329	AAP55251.1	AY220730.1/ 25643-26779	Mol. Microbiol. 2003; 49(1):193-210.
<i>S. aureus</i> RF122	WP_000179355.1	NC_007622.1/ 2037180-2038352	PLoS One. 2007; 2(10):e1120.
<i>S. pseudintermedius</i> 081661	WP_037541925.1	CP016073.1/ 2667235-2668371	Direct Submission: Riley, Bemis and Kania, 2016.
<i>S. aureus</i> CS6-EEFIC (SaRIfusB)	CAL23817.1	AM292600.1/ 2580-1405	J. Clin. Microbiol. 2007; 45(5):1505-10. Epub 2007 Mar 7.
<i>S. aureus</i> Mu50 (SaPlm1)	WP_000179343.1	NC_002758.2/ 2147530-2148702	Lancet. 2001; 357(9264):1225-40.
<i>S. aureus</i> Mu50 (Tn5801)	BAB56554.1	NC_002758.2/ 437264-436071	Lancet. 2001; 357(9264):1225-40.
<i>S. aureus</i> Mu50 (SaPlm4)	WP_000121223.1	NC_002758.2/ 869568-868462	Lancet. 2001; 357(9264):1225-40.
<i>S. aureus</i> FORC_001	WP_000179345.1	NZ_CP009554.1/ 2161377-2162549	Microbiology. 2007; 153(Pt 10):3235-45.
<i>B. subtilis</i> 168	NP_388361.1	NC_000964.3/ 530611-529505	Proc. Natl. Acad. Sci. U. S. A. 2004; 101(26):9786-91.
<i>S. aureus</i> MRSA252	WP_000270135.1	NC_002952.2/ 411390-410176	Microbiol. Immunol. 2013; 57(2):91-9.
<i>S. aureus</i> IMV10	BAM66883.1	AB716349.1/ 2727-1510	Direct Submission: Muzny et al., 2010
<i>S. aureus</i> TCH60	ADQ77825.1	CP002110.1/ 2475780-2477024	Mol. Microbiol. 1998; 29(2):527-43.
<i>S. aureus</i> RN4282	AAC28969.1	U93688.2/ 13875-15095	J. Biol. Chem. 2002; 277(15):13138-47.
<i>S. aureus</i> COL	AAL04148.1	AF410775.1/ 14574-15794	Lancet. 2002; 359(9320):1819-27.
<i>S. aureus</i> MW2	WP_025174195.1	NC_003923.1/ 840811-839705	Antimicrob. Agents Chemother. 2013; 57(11):5737-9.
<i>S. epidermidis</i> NTUH-857	BAO09125.1"	AB828060.1/ 2254-3911	Plasmid. 1994; 32(3):350-4.
<i>E. faecalis</i> (Tn916)	AAB60030.1	U09422/ 16641-17858	

Supplementary Table S4. Excision of circular DNA intermediates from *M. caseolyticus* chromosome.

Template	PCR product	PCR primers	Elongation time	Amplicon size/bp	Recombination sites	DR present in amplicon
Circular intermediate						
IMD0819	McRI _{mecD-1}	int-0819-F and mecD-F	15 min	9,970	DR1 and DR2	DR1
IMD0819	McRI _{mecD-1} -McCI _{IMD0819}	int-0819-F and mecD-F	15 min	12,563	DR1 and DR3	DR1
IMD0819	McCI _{IMD0819}	orf21-R and araC-F	1 min	763	DR2 and DR3	DR2
KM0211	McRI _{mecD-1}	int-0819-F and mecD-F	15 min	9,970	DR1 and DR2	DR1
IMD0473	none	int-0473-F and mecD-F	7 min	-	-	-
Chromosomal segment after excision						
IMD0819	Δ McRI _{mecD-1}	truA-F and cop-R	7 min	7,075	DR1 and DR2	DR2
IMD0819	Δ McRI _{mecD-1} - Δ McCI _{IMD0819}	truA-F and cop-R	7 min	4,301	DR1 and DR3	DR3
IMD0819	Δ McCI _{IMD0819}	orf20-F and cop-R	2 min	4,158	DR2 and DR3	DR3
KM0211	Δ McRI _{mecD-1}	truA-F and cop-R	1.5 min	2,224	DR1 and DR2	DR2