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A *Staphylococcus xylosus* Isolate with a New *mecC* Allotype

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Recently, a novel variant of *mecA* known as *mecC* (*mecA*$_{LGA251}$) was identified in *Staphylococcus aureus* isolates from both humans and animals. In this study, we identified a *Staphylococcus xylosus* isolate that harbors a new allotype of the *mecC* gene, *mecC1*. Whole-genome sequencing revealed that *mecC1* forms part of a class E *mec* complex (*mecI-mecR1-mecC1-blaZ*) located at the *orfX* locus as part of a likely staphylococcal cassette chromosome *mec* element (SCC*mec*) remnant, which also contains a number of other genes present on the type XI SCC*mec*.

Methicillin resistance in staphylococci is encoded by *mecA*, encoding the penicillin-binding protein 2a (PBP2a), which has a low affinity for beta-lactam antibiotics (1). As a result, the transpeptidase activity of PBP2a is functional at normally inhibitory concentrations of beta-lactam antibiotics, allowing cell wall synthesis to occur (2–4). Recently, a novel variant of *mecA* was identified in *Staphylococcus aureus* from cattle (5), humans, and a range of other animal species (6) in Denmark, France, The Netherlands, Ireland, Germany, Belgium, and the United Kingdom (5, 7–11). This subtype was originally designated *mecA*$_{LGA251}$ but has since been renamed *mecC* and shares 70% nucleotide identity with the conventional *mecA* gene. The *mecC* gene is present with its cognate regulators *mecI-mecR1*, as part of a class E *mec* complex that shares structural similarity (*mecI-mecR1-mecC-blaZ*) with a *mec* gene complex found in *Macrococcus caseolyticus* (12). The class E complex is present as part of a larger, 29.4-kb, type XI staphylococcal cassette chromosome *mec* element (SCC*mec*) inserted at *orfX*; this element also includes the recombinase genes *ccrAB* and arsenic resistance genes. In this work, we describe a highly related *mecC* homolog present in the *orfX* locus in a *Staphylococcus xylosus*.

A search of the EMBL nucleotide database identified submission of sequences from *S. xylosus* strain S04009 (13) with a high degree of similarity (>90%) to *mecC* (5, 8). However, antimicrobial susceptibility testing of strain S04009 by disk diffusion with oxacillin and cefoxitin showed it to be susceptible to both antibiotics but resistant to penicillin using British Society for Antimicrobial Chemotherapy (BSAC) criteria (version 10.2) (data not shown). Therefore, we submitted the *mecC*-positive strain S04009 and a *mecC*-negative *S. xylosus* isolate, S040010, for whole-genome sequencing to further characterize the *mecC*-containing region. Illumina library preparation was carried out as described by Quail et al. (14), and HiSeq sequencing was carried out following the manufacturer's standard protocols (Illumina, Inc.). Genome sequencing confirmed the presence of a *mecC* homolog in S04009 located downstream of the *S. xylosus orfX* homolog, a region associated with horizontally transferred elements (Fig. 1). Immediately downstream of *orfX* in S04009 is a 3.3-kb region that shows a high degree of similarity (>95% nucleotide identity) to the 3′ end of the arginine catabolic mobile element (ACME) in the *S. aureus* USA300 strain FPR3757 (EMBL accession no. CP000255) and contains a truncated version of *copA*, an ATPase copper transporter (15). Next to this region is the *mec* complex. The *mecC* gene in S04009 shares 93.5% nucleotide identity to *mecC* in *S. aureus* LGA251 and 69.9% to *mecA* from *S. aureus* strain MRSA252. Based on the current guidelines for reporting *mecA* homologs, the S04009 *mecC* gene is a new allotype of the LGA251 *mecC*, herein referred to as *mecC1* (16). Sequence analysis of the *mecC1* gene identified a frameshift mutation close to the 5′ end of the gene, resulting in a truncated 64-amino-acid (aa) product, providing a molecular basis for the oxacillin and cefoxitin susceptibility of strain S04009. *mecC1* is found in a homologous class E *mec* gene complex (*mecI-mecR1-mecC1-blaZ*) which has been previously reported in *S. aureus* (5, 8) The presence of the *blaZ* gene is likely to account for the observed penicillin resistance of S04009 despite *mecC1* being inactivated. *mecI*, *mecR1*, and *blaZ* in S04009 share 91.1%, 90.0%, and 90.9% nucleotide identity, respectively, with their homologs in LGA251. Downstream of the *mec* gene complex is a hypothetical protein conserved in a number of coagulase-negative *Staphylococcus* (CoNS) species, followed by a tandem pair of ATP-binding cassette transporters (ABC transporters). After the final ABC transporter gene, there is an imperfect 53-bp inverted repeat (IR), which suggests that this region was once part of a separate mobile element or has undergone deletion mediated by this repeat. Immediately upstream of this is a *myo*-inositol (MI)

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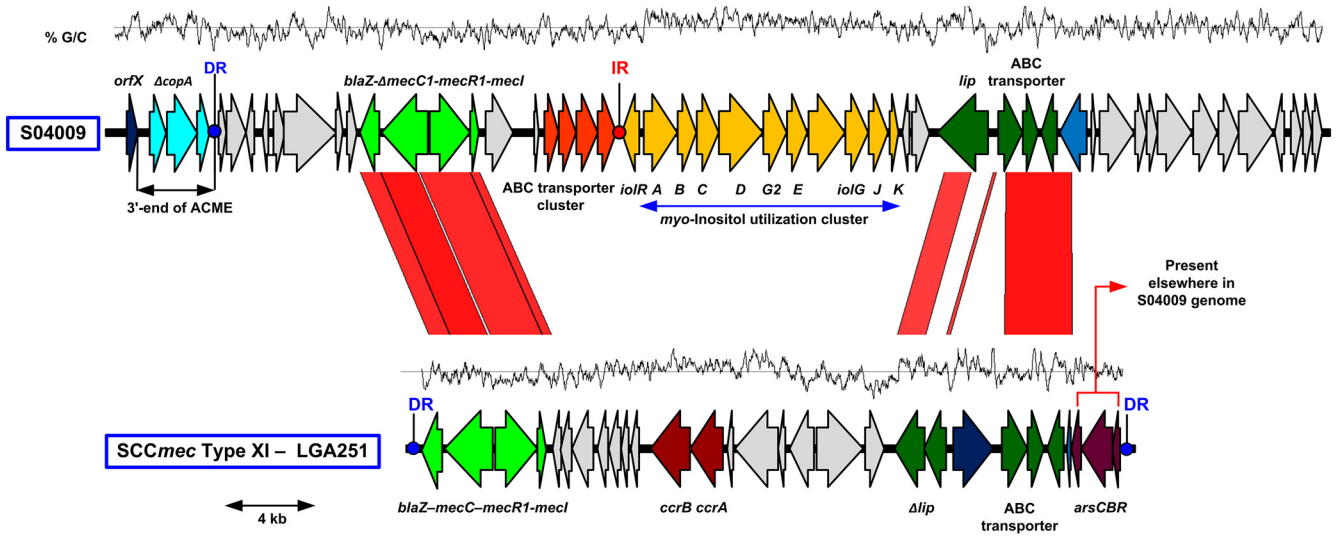

FIG 1 Comparison of the *orfX* region in *S. xylosus* S04009 (EMBL accession no. HE993884) and the SCC*mec* type XI in LGA251 (EMBL accession no. FR821779). Red areas are regions conserved between the two sequences, and homologous coding sequences (CDSs) are in bright and dark green. Other CDSs of interest discussed in the text are highlighted in color. Blue and red dots indicate direct repeats (DR) and inverted repeats (IR), respectively. The GC content is shown above each region.

utilization cluster, which was previously identified in strain S04009 by subtractive hybridization (13). Downstream from the MI utilization cluster are more genes present in joining region 1 (J1) in the type XI SCC*mec* in LGA251. The lipase gene, which is present as two truncated pseudogenes (SARLGA251_00420 and SARLGA251_00430) in LGA251, is intact in S04009. Adjacent to this are genes for an ABC transporter permease, an ABC transporter ATPase, and a conserved hypothetical protein (SARLGA251_00470-490) with 96%, 97%, and 98% nucleotide identity, respectively, to those in LGA251. Downstream of the conserved ABC transporter genes in S04009 is a gene for a major facilitator superfamily (MFS) protein that is absent from SCC*mec* type XI, ending the region of homology. Interestingly, *arsR*, *arsB*, and *arsC* are present in S04009 and share 83%, 88%, and 91% nucleotide identity, respectively, with their homologs in LGA251. However, they are not found proximal to the *orfX* region but are instead associated with a Tn*554*-like transposon and are inserted at a different location in the S04009 genome (data not shown).

In order to further understand the evolutionary history of the *mecC1*-containing element in *S. xylosus*, we compared the *orfX* locus of strain *S. xylosus* S04009 with those of two other *S. xylosus* strains, S040010, and a third *S. xylosus* strain, C2a (S. Leroy, unpublished data). Immediately downstream of the *orfX* in C2a is an ~9-kb region absent from S04009, which shares blocks of homology to *Enterococcus faecalis* D32 (EMBL accession no. CP003726) at the 5′ end and to *Staphylococcus haemolyticus* JCSC1435 (EMBL accession no. AP006716) at the 3′ end. This region contains a number of genes associated with mobile elements, including a truncated abortive phage infection protein (AIPR), a type I restriction modification system restriction subunit, and two genes that likely encode an McrBC 5-methylcytosine restriction system. Immediately flanking this region is a truncated copy of the putative Na^+/*myo*-inositol cotransporter, which is interrupted by a 55-bp imperfect inverted repeat (Fig. 2). In S04010, downstream of *orfX* is an ~30 kb region which is absent from both S04009 and C2a. This region displays short regions of homology to corresponding regions in *E. faecalis*, *Staphylococcus carnosus* subsp. *carnosus*, *S. aureus*, and a number of other Gram-positive species. The region proximal to *orfX* contains a number of hypothetical proteins and, like C2a, a putative restriction modification system. Downstream of this is a sorbitol utilization operon which is found next to a type IV SCC*mec* in *S. aureus* strain VRS3a (17) and part of a SCC*mec*WAMRSA40 composite island (EMBL accession no. JQ746621) which is found on the chromosome in *S. carnosus* strain TM300 (17). The sorbitol operon is also present in *E. faecalis* strain D32. Further downstream from this are three genes that make up a *bgl* (aryl-β,D-glucoside) operon. Downstream from this is an ~200-bp region that shares 91% nucleotide identity with the IR-containing region in C2a (Fig. 2), the IR itself being identical in 50 of 55 nucleotides. Further small regions of homology exist between S04009 and S04010, consisting of an ~750 bp region immediately downstream of the ACME DR in S04009 and a region just before the *bgl* operon in S04010. In order to ascertain the prevalence of *mecC1* in *S. xylosus* strains, we screened a total of 114 *S. xylosus* isolates from a wide range of sources, though with a deliberate bias toward isolates from bovine milk, as this was the original source of the strain S04009 (Table 1). (Additional information about *S. xylosus* strains screened for *mecC* is presented in Table 2.) We screened the strains by PCR using primers for *mecC*/*mecC1*, *blaZ*, and *mecA* and universal staphylococcal 16S primers (Table 3). Neither *mecA*, *mecC*, nor *blaZ* was detected in any of these isolates.

The finding that multiple components of the type XI SCC*mec* are present in contiguous blocks in the chromosome of *S. xylosus* S04009 suggests that this element may represent the remnants of an ancestral SCC*mec* element. Given the lack of any SCC*mec* flanking repeats in S04009 and the change in the GC content after the inverted repeat between the MI utilization cluster region and the *mecC1*-containing region, it is not clear if these two regions represent a single larger element or multiple independent acquisitions by an ancestral strain. The presence of the truncated MI cluster in C2a does suggest that the MI utilization cluster was part

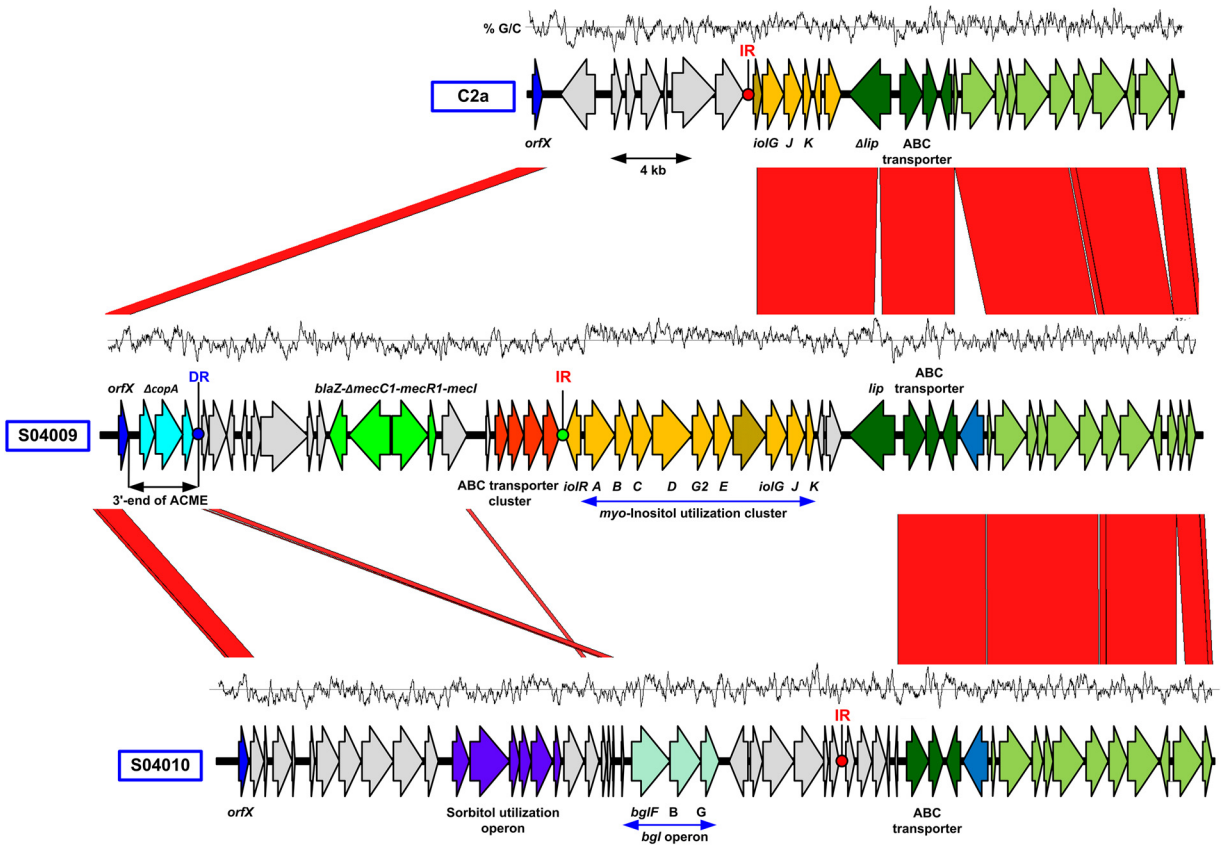

FIG 2 Comparison of the *orfX* regions in *S. xylosus* strains C2a (EMBL accession no. HE993886), S04009 (EMBL accession no. HE993884), and S04010 (EMBL accession no. HE993885). Red areas show regions conserved between the two sequences, and homologous CDSs are in light and dark green. Other CDSs of interest discussed in the text are highlighted in color. Blue dots indicate direct repeats (DR), and green and red dots show inverted repeats (IR) (the inverted repeats in C2a and S04010 are virtually identical). The GC content of the region is shown above each genome schematic. The eighth gene in the *myo*-inositol cluster that is truncated in C2a and intact in S04009 is indicated with shading.

of a single contiguous block with the lipase and the ABC transporters in both S04009 and C2a. The finding that the arsenic resistance genes are also present in S04009 in association with a transposon further highlights a potential mechanism for the acquisition of these genes into the type XI SCC*mec*. Therefore, based on the available evidence, we suggest that the class E *mec* complex in *S. xylosus* was part of a larger ancestral SCC*mec* element which probably included the MI cluster and the lipase and ABC transporters and that this element has undergone gradual deletion and acquisition (of the arsenic resistance genes) to the type XI SCC*mec* identified in *S. aureus* LGA251 (5). The fact that we found no other *S. xylosus* strains harboring *mecC* or *blaZ* suggests that *mecC1* might be present in only a minor subset of *S. xylosus* isolates. It is noteworthy that *S. xylosus* is present in fermented foods such as sausage (18, 19) and cheese (20), highlighting another potential route for the transmission of antibiotic resistance genes from the environment to human flora (21). Given the recent discovery of *mecR2*, a third regulator of *mecA* expression, it would also be interesting to see if the expression of *mecC1* is positively regulated in the same way by *xylR*, encoding the xylose operon repressor (present in *S. xylosus* S04009), which is a close homolog of the *mecR2* regulator (22). In *S. xylosus*, as in other staphylococci, the *orfX* locus is a site for the integration of multiple SCC-like elements. The strains analyzed in this study have metabolic utilization clusters present at *orfX*, which may reflect the biological niche occu-

TABLE 1 Bacterial strains used in this study

Species	Strain	Relevant characteristics	Reference
S. xylosus	S04009	*mecC1*, bovine mastitis	13
S. xylosus	S040010	Bovine mastitis	13
S. xylosus	C2a	Human skin commensal	13
S. aureus	LGA251	*mecC*, ST425	5

TABLE 2 Overview of *Staphyloccocus xylosus* isolates screened for *mecC*

No. of isolates screened	Relevant characteristics	Reference
15	The Netherlands, bovine milk; oxacillin MIC ≥ 0.5 μg/ml	26
20	Switzerland, bovine milk; oxacillin MIC ≥ 0.5 μg/ml	This work
5	France, various sources	13
3	Switzerland, horse skin; 2 isolates with oxacillin MIC ≥ 0.5 μg/ml	27
70	United States, bovine milk and streak canals	28 and this work
1	United States, human skin; ATCC 29971	29

TABLE 3 Oligonucleotide primers used in this study

Primer name	Sequence (5′→3′)	Target	Reference
mecC1 + 2_F	5′-AAGTTAATCAAAAATGGGTTCAGC-3′	*mecC*	This work
mecC1 + 2_R	5′GGTTGTAATGCTGTACCAGATCC-3′	*mecC*	This work
blaZ_XI_F	5′-CGTTTTGCWTATGCTTCCAC-3′	*blaZ*	This work
blaZ_XI_R	5′-CKGGTTCTTTTCTAGATGGATG-3′	*blaZ*	This work
MecA1	GTA GAA ATG ACT GAA CGT CCG ATA A	*mecA*	30
MecA2	CCA ATT CCA CAT TGT TTC GGT CTA A	*mecA*	30
16SF	CCTATAAGACTGGGATAACTTCGGG	16S rDNA	31
16SR	CTTTGAGTTTCAACCTTGCGGTCG	16S rDNA	31

pied by the *S. xylosus* isolates included in this study. In addition, regions of DNA are present in both C2a and S40010 with close homology to *E. faecalis* strain D32, an isolate from a pig (23). This indicates that horizontal gene transfer between enterococci and staphylococci is a relatively common occurrence (24, 25), an important observation in relation to the transfer of vancomycin resistance to *S. aureus*. In conclusion, this study further highlights the fact that CoNS from both humans and animals are an important reservoir of resistance genes that have the potential to be transferred into more pathogenic staphylococcal species.

Nucleotide sequence accession numbers. The nucleotide sequences determined for the *orfX* region of S04009, S04010, and C2a have been deposited in the EMBL database under accession numbers HE993884, HE993885, and HE993886, respectively.

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