Genome Sequence of *Staphylococcus equorum* subsp. *equorum* Mu2, Isolated from a French Smear-Ripened Cheese

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Staphylococcus equorum subsp. *equorum* is a member of the coagulase-negative staphylococcus group and is frequently isolated from fermented food products and from food-processing environments. It contributes to the formation of aroma compounds during the ripening of fermented foods, especially cheeses and sausages. Here, we report the draft genome sequence of *Staphylococcus equorum* subsp. *equorum* Mu2 to provide insights into its physiology and compare it with other *Staphylococcus* species.

Members of the *Staphylococcus* genus are ubiquitously distributed in nature. They are found on the skin and mucous membranes of warm-blooded animals, including humans, and are also isolated from a wide range of foodstuffs and environmental sources (10). Coagulase-negative staphylococci (CNS) constitute one of the main groups responsible for the flavor intensity of cheeses and fermented sausages (1, 2, 3, 4, 7, 9).

Studies have shown that two subspecies of *Staphylococcus equorum* are the dominant species among staphylococcal isolates from fermented sausages found in food-processing environments (6, 12, 13) and on the surface of cheeses, and they can be used as an ingredient in starter cultures for smear-ripened and semihard cheeses (2, 17).

Historically, no reported incidents of staphylococcal food poisoning, including those caused by milk products, have been attributed to CNS. However, this view is changing and there is increasing evidence that some CNS may be pathogenic for humans through enterotoxin production or may cause nosocomial infections (16). Since the prevalence and pathogenic potential of CNS have not been specifically studied, it is necessary to assess the safety status of these organisms in order to use them as components of starter cultures for fermented foods.

The genome sequence of *Staphylococcus equorum* subsp. *equorum* Mu2, isolated from a smear-ripened cheese, was determined by Roche GS-FLX Titanium series chemistry with a long paired-end library protocol. Genomic libraries containing 8-kb inserts were constructed, and 692,501 reads were assembled with Newbler, which generated eight scaffolds (30 contigs) greater than 500 bp with a coverage of 47-fold. Genome annotation was performed by AGMIAL (5), an integrated bacterial genome annotation system. Prediction of coding sequences used SHOW, the self-training gene detection software based on hidden Markov models (<http://genome.jouy.inra.fr/ssb/SHOW/>). tRNA and rRNA were detected using tRNAscan-SE (14) and RNAmmer (11) software, respectively. Web-based software and databases were used to manually curate predicted genes after comparing data to those in public databases (COGs [18], conserved domain database [15], and InterPro [8]). The draft genome is 2,927,171 bp in length, with a G+C content of 32.8%, and includes 2,781 predicted coding sequences and 129 pseudogenes. Biological functions could be defined for 1,823 (65.6%) of the predicted proteins.

Comparative genomic analyses with strains belonging to other *Staphylococcus* species revealed that the *S. equorum* subsp. *equorum* Mu2 genome shares some similarities with the *S. saprophyticus* genome: it does not encode any of the virulence factors found in *S. aureus*. Interestingly, unlike most staphylococci, *S. saprophyticus*, *S. intermedius*, and *S. equorum* subsp. *equorum* Mu2 do not possess a lactose-specific phosphotransferase system (PTS) or a phospho- β -galactosidase. They do not have the genetic potential to degrade galactose by the tagatose-6-phosphate pathway, but the genes encoding the enzymes of the Leloir pathway are present.

A detailed analysis of this genome and comparative genome analyses with other *Staphylococcus* species will help to identify the specific features of this technological food-grade strain.

Nucleotide sequence accession numbers. The genome sequence and annotation of *Staphylococcus equorum* subsp. *equorum* Mu2 have been deposited in the EMBL database under the accession numbers [CAJL01000001](#) to [CAJL01000030](#).

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REFERENCES

1. Blaiotta G, et al. 2004. Diversity and dynamics of communities of coagulase-negative staphylococci in traditional fermented sausages. *J. Appl. Microbiol.* 97:271.
2. Bockelmann W. 2002. Development of defined surface starter cultures for the ripening of smear cheeses. *Int. Dairy J.* 12:123–131.
3. Bockelmann W, et al. 1997. The microflora of Tilsit cheese. Part 2. Development of a surface smear starter culture. *Nahrung* 41:213–218.
4. Bockelmann W, Hoppe-Seyler T. 2001. The surface flora of bacterial smear-ripened cheeses from cow's and goat's milk. *Int. Dairy J.* 11:307–314.

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5. Bryson K, et al. 2006. AGMIAL: implementing an annotation strategy for prokaryote genomes as a distributed system. *Nucleic Acids Res.* 34:3533–3545.
6. Corbière Morot-Bizot S, Leroy S, Talon R. 2006. Staphylococcal community of a small unit manufacturing traditional dry fermented sausages. *Int. J. Food Microbiol.* 108:210–217.
7. Hoppe-Seyler T, Jaeger B, Bockelmann W, Heller KJ. 2000. Quantification and identification of microorganisms from the surface smear cheeses. *Kieler Milchw. Forsch.* 52:294–305.
8. Hunter S, et al. 2009. InterPro: the integrative protein signature database. *Nucleic Acids Res.* 37:211–215.
9. Irlinger F, Morvan A, El Solh N, Bergère JL. 1997. Taxonomic characterization of coagulase-negative staphylococci in ripening flora from traditional French cheeses. *Syst. Appl. Microbiol.* 20:319–328.
10. Kloos WE, Schleifer KH. 1986. Genus IV. *Staphylococcus*, p 1013–1035. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (ed), *Bergey's manual of systematic bacteriology*. Williams & Wilkins, Baltimore, MD.
11. Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108.
12. Leroy S, et al. 2010. Biodiversity of indigenous staphylococci of naturally fermented dry sausages and manufacturing environments of small-scale processing units. *Food Microbiol.* 27:294–301.
13. Leroy S, et al. 2009. Genetic diversity and biofilm formation of *Staphylococcus equorum* isolated from naturally fermented sausages and their manufacturing environment. *Int. J. Food. Microbiol.* 134:46–51.
14. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
15. Marchler-Bauer A, et al. 2009. CDD: specific functional annotation with the conserved domain database. *Nucleic Acids Res.* 37:D205–D210.
16. Martineau F, et al. 2000. Development of a rapid PCR assay specific for *Staphylococcus saprophyticus* and application to direct detection from urine samples. *J. Clin. Microbiol.* 38:3280–3284.
17. Place RB, Hiestand D, Gallmann HR, Teuber M. 2003. *Staphylococcus equorum* subsp. *linens*, subsp. nov., a starter culture component for surface ripened semihard cheeses. *Syst. Appl. Microbiol.* 26:30–37.
18. Tatusov RL, et al. 2003. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* 4:41.