A Coagulate- and α-Glucosidase-Negative Variant of Staphylococcus aureus: a Challenge for Routine Microbiological Diagnostics

Staphylococcus aureus can cause serious infections, toxicoses, and life-threatening illnesses. Staphylococcal coagulase production represents the major criterion for the detection of S. aureus isolates. As coagulase-deficient clinical isolates of S. aureus have been described, additional use of chromogenic media in S. aureus detection was postulated to represent a highly specific (97%) and sensitive (99% after 48 h) tool in the identification of the organism (1, 3, 4, 7, 8, 10). In this study, we further investigated the genotypic and phenotypic characteristics of a coagulase- and α-glucosidase-deficient variant of S. aureus obtained from bovine mastitis milk.

Strain MSSA_129 (methicillin-susceptible S. aureus) was identified as S. aureus by species-specific PCR, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) (99.90% identification), and DNA microarray and could be assigned to spa type 1543, clonal complex CC479, agr type II, and capsule type 8. It exhibited alpha, beta, and delta hemolysis and was slightly yellow pigmented. Staphaureux (Thermo Fisher Scientific, Wohlen, Switzerland), DNase, and egg yolk test yielded positive results. Still, no opaque zone was visible on rabbit plasma fibrinogen (RPF) agar (Oxoid, Cambridge, United Kingdom) (Fig. 1). On S. aureus ID agar (SAID; bioMérieux, La Balme les Grottes, France), bright yellow colonies instead of the characteristic green colonies were formed (Fig. 2). DNA microarray detected neither antibiotic resistance determinants nor genes involved in toxic shock and staphylococcal scalded-skin syndrome. While the classical enterotoxin genes (sea, seb, sec, sed, and see) were not detected, the strain exhibited the egc cluster carrying genes encoding newly described staphylococcal enterotoxins and staphylococcal enterotoxin-like superantigens.

Determination of the nucleotide sequence of coa revealed a deletion at nucleotide position 653 within the D2 region of the gene, leading to a frameshift. Although coa is known to represent a highly polymorphic region, it can be divided into six common regions: the signal sequence, the D1 and D2 regions enabling contact with prothrombin, the central region, a repeat region, and the C-terminal sequence (5, 9, 11, 12). The frameshift detected results in the reading of a premature stop codon at amino acid position 224, thus rendering the polypeptide abnormally short and most likely not functional.

The α-glucosidase gene of MSSA_129 and its upstream region were compared to sequences available at GenBank. Although no strain possesses an α-glucosidase gene exactly identical over its full length to the one found in MSSA_129, no unique amino acid changes were found. However, variability of amino acid sequences on 12 positions (position 43, 83, 88, 133, 439, 448, 454, 462, 492, 514, 519, and 539) within the available sequences was noticed. Considering the amino acids found at these positions, strain MSSA_129 showed a unique combination. Directly upstream of the α-glucosidase gene (malA), we identified a sequence homologous to malR, a putative transcriptional regulator involved in maltose transport in Staphylococcus xylosus. In S. xylosus, the malRA genes are cotranscribed and constitute an essential locus for maltosaccharide utilization (2). MSSA_129 malR exhibited several unique amino acid changes. The expression levels of malA were compared among MSSA_129 and two phenotypically α-glucosidase-positive strains, LRA1 and HG003 (6), by quantitative real-time PCR. As MSSA_129 expressed malA at levels similar to those in HG003 and even at significantly higher levels than in LRA1 (P = 0.01), the hypothesis that the yellow phenotype may be due to a negative effect of malR polymorphisms on malA expression was dismissed. Complementation of MSSA_129 with the α-glucosidase gene of strain HG003 on a plasmid rescued the green phenotype on SAID agar. Complementation of MSSA_129 with its own malA or an empty vector did not alter the phenotype on SAID. We therefore suggest the atypical phenotype on SAID to be due to the sequence polymorphisms discovered in the MSSA_129 α-glucosidase gene.

Considering our findings, screening for S. aureus by only RPF and SAID agar harbors the risk of false-negative results, potentially leading to severe therapeutic mistakes.

Materials and Methods, as well as DNA microarray results, are in the supplemental material.

Nucleotide sequence accession numbers. Nucleotide sequences of genes encoding staphylococcal coagulase (coa), as well as α-glucosidase (malA) and its upstream region in the investigated strain MSSA_129, were deposited in GenBank (accession numbers JN861807, JN861808, and JQ403276).
FIG 2 The SAID plate on the left shows characteristic green colonies formed by a phenotypically α-glucosidase-positive S. aureus strain. The SAID plate on the right shows the distinctive bright-yellow colonies formed by the atypical S. aureus isolate MSSA_129 investigated in this study.

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