A coagulase and α-glucosidase negative variant of *Staphylococcus aureus* – a challenge for routine microbiological diagnostics

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* Staphylococcus (S.) aureus* can cause serious infections, toxinses and life-threatening illnesses. Staphylocoagulase production represents the major criterion for 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 48h) tool in the identification of the organism (1, 3, 4, 7, 8, 10). In this study, we further investigated genotypic and phenotypic characteristics of a coagulase and α-glucosidase deficient variant of *S. aureus* obtained from bovine mastitis milk.

Nucleotide sequences of genes encoding staphylocoagulase (*coa*), as well as α-glucosidase (*malA*) and its upstream region in the investigated strain MSSA_129 were deposited in Genbank.
MSSA_129 was identified as *S. aureus* by species-specific PCR, MALDI-TOF (ID 99.90%), and DNA microarray, and could be assigned to *spa* type t543, 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 agar (RPF, Oxoid, Cambridge, United Kingdom) (see Figure 1). On *S. aureus* ID agar (SAID, Biomérieux, La Balme les Grottes, France), bright yellow colonies instead of the characteristic green colonies were formed (see Figure 2). DNA microarray detected neither antibiotic resistance determinants, nor genes involved in toxic shock and staphylococcal scalded skin syndrome. While no classical staphylococcal enterotoxins SEA-SEE were 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 detected frameshift 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 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 malsaccharide utilization (2). MSSA_129 *malR* exhibited several unique amino acid changes. 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* in similar levels compared to HG003, and even in 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.
FIGURE 1: While colonies of a strain LRA1 (left plate) form a characteristic opaque halo on RPF agar, the atypical S. aureus strain MSSA_129 (right plate) lacks this feature.
Figure 2: The SAID plate on the left side shows characteristic green colonies formed by a phenotypically α-glucosidase-positive *S. aureus* strain. The SAID plate on the right side shows the distinctive bright-yellow colonies formed by the atypical *S. aureus* isolate MSSA_129 investigated in this study.

REFERENCES


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