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The Catalase Gene Differentiates between Some Strains of *Staphylococcus aureus* ssp. *anaerobius*

N.O. MUSA[a,b‡], K. ELTOM[a,b*‡], F. GESSLER[a**], H. BÖHNEL[a**], A. BABIKER[c], S.M. EL SANOUSI[c]

[a]*Institute of Tropical Animal Health, Georg-August University of Göttingen, D-37077 Göttingen, Germany*
[b]*Institute for Promotion of Animal Export Studies, University of Khartoum, 13314 Shambat, Khartoum North, Sudan*
[c]*Faculty of Veterinary Medicine, University of Khartoum, 13314 Shambat, Khartoum North, Sudan*

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ABSTRACT. *Staphylococcus aureus* ssp *anaerobius* strain S10 was isolated from an outbreak of sheep abscess disease. Sequence of the catalase gene of this strain showed 99 % identity to the catalase gene (*katB*) sequence of the reference strain (*S. aureus* ssp. *anaerobius* strain MVF213) with mismatching of three base pairs. An important substitution located 1036 nucleotides upstream of the initiation codon from "C" in *katB* to "T" in the catalase gene of strain S10 originated a stop codon. The deduced protein (345 amino acids) is 105 amino acids shorter than that of *katB*. Partial sequence of the catalase gene of other 8 local isolates in addition to another reference strain (DSM 20714/ATCC 35844) revealed the same mutations in all local (African) strains, whereas the sequence of the reference (European) strain was typical to that of *katB*. Sequence of the catalase gene of *S. aureus* ssp. *anaerobius* strain S10 was deposited in *GenBank* under accession no. EU281993.

Abbreviations

katA	catalase gene of *Staphylococcus aureus*
katB	catalase-like protein gene of *Staphylococcus aureus* ssp. *anaerobius* MVF213
katS10	catalase-like protein gene of *Staphylococcus aureus* ssp. *anaerobius* strain S10
SaanS10	*Staphylococcus aureus* ssp. *anaerobius* strain S10

Anaerobic *Staphylococcus aureus* bacteria are the causal agent of sheep abscess or Morel's disease (Bajmócy *et al.* 1984; Hamad *et al.* 1992). Although these bacteria are considered nonpathogenic for humans, a report on a case of septicemia due to one strain of these bacteria in man has recently been published (Peake *et al.* 2006). These bacteria were separated from other *S. aureus* bacteria in a subspecies (*S. aureus* ssp. *anaerobius*) because of their negative or weak growth in normal air, lack of the catalase activity in addition to some other biochemical properties (de la Fuente *et al*. 1985). Although some strains of *S. aureus* ssp. *aureus* were reported to lack this catalase enzyme activity (Tu *et al*. 1976; Friedberg *et al*. 2003; Yilmaz *et al*. 2005), they still grow well under aerobic conditions (Grüner *et al*. 2007).

Comparative studies between the catalase genes of *S. aureus* ssp. *aureus* and *S. aureus* ssp. *anaerobius* (*katA* and *katB*, respectively) showed that *katA* had undergone mutations leading to deletion of one base pair in addition to 8 silent and 6 mis-sense mutations (Sanz *et al*. 2000). The deletion resulted in shift of the reading frame and premature termination of translation with subsequent generation of *katB*, which codes for a protein 55 amino acid residues shorter than *katA*. Lack of the catalase activity of *S. aureus* ssp. *anaerobius* is attributed to some of these mutations (Sanz *et al*. 2000). The loss of catalase activity in some strains of *S. aureus* ssp. *aureus* was also attributed to mutations of the catalase gene (*katA*). While in a methicillin-resistant *S. aureus* ssp. *aureus* strain deletion of five successive base pairs led to a shift in the reading frame and premature termination of translation (Grüner *et al*. 2007), substitution of a key amino acid in the protein (His58Tyr) led to inactivity of this gene in methicillin-sensitive *S. aureus* ssp. *aureus* strain (Piau *et al*. 2008).

The aim of this investigation was to characterize by molecular means some *S. aureus* ssp. *anaerobius* strains isolated from animals. We conducted full sequencing of the catalase gene of one isolate from an outbreak of sheep abscess disease in addition to partial sequencing of this gene for nine other isolates including one reference strain. We report here on some strains of *S. aureus* ssp. *anaerobius* harboring a catalase gene that underwent mutations other than those previously reported for the European strains.

**Corresponding author*; Institute for Promotion of Animal Export Studies, University of Khartoum, 13314 Shambat, Khartoum North, Sudan; fax +249 185 326 827, e-mail keltom@daad-alumni.de.

***Present address*: Institute of Applied Biotechnology in the Tropics at Georg-August University of Göttingen, Marie-Curie-Str. 7, 37079 Göttingen, Germany.

‡These authors contributed equally to the paper.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* ssp. *anaerobius* strain S10 (SaanS10) was isolated from superficial lymph node abscess of one lamb in a flock of sheep during outbreak of Morel's disease in Alsamra village (East Nile Province of the Sudan) (Musa *et al*. 2007). Other isolates and strains used were: two isolates from animals in the same disease outbreak, 6 isolates from superficial lymph node abscesses of sheep at meat inspection in abattoirs located in two different areas of Khartoum State, and *S. aureus* ssp. *anaerobius* DSM 20714/ ATCC 35844 as a reference strain. Identification of the isolates was based on a failure of aerobic growth within 2 d, lack of catalase activity, positive coagulase activity, in addition to the fermentation ability of some sugars. All tests were done according to standard methods (Barrow and Feltham 1993).

DNA extraction. Genomic DNA was extracted using Axy Prep Bacterial Genomic DNA Miniprep Kit of Axygen (*Bioron*, Germany) with some modifications of the manufacturer's protocol. In brief, 3–5 colonies from a 2-d blood agar culture were suspended in 150 µL of the recommended buffer. Lysis of the cells was achieved by treatment with 10 µL of 1 % lysostaphin (*Sigma*, Germany) for 1 h at 37 °C followed, after addition of 2 µL 10 % Proteinase K (*Bioron*), by a 2-h treatment at 56 °C. The follow-up steps were carried out according to the manufacturer's protocol.

PCR. To confirm the biochemical identification of the isolates, a conserved region of the thermonuclease gene (*nuc* gene) of *S. aureus* was amplified by PCR using primers and conditions described by Brakstad *et al*. (1992).

Sequencing of the catalase gene. In order to amplify and sequence the whole catalase gene of SaanS10 and to partially sequence the catalase gene of the other isolates, primers and conditions previously described for the amplification of *katA* and *katB* (Sanz *et al*. 2000) in addition to other primers designed for this purpose were used (Table I). Sequencing was done by *Seqlab* (Göttingen, Germany). For confirmation of the sequence results, both strands were sequenced, or overlapping parts of the gene were sequenced. Sequences were edited using a software program (BioEdit, Version 7.0.5.3). Alignment and comparisons were done using the Basic Local Alignment Search Tool (BLAST) of NCBI. The resulting sequence of the catalase gene of SaanS10 was deposited in the *GenBank* under accession no. EU281993; those of the other 8 local isolates and the reference strain were deposited under accession nos FJ935782–FJ935790.

Table I. Oligonucleotides used

Primer	Sequence (5′→3′)	Gene	Source
Nuc F	GCG ATT GAT GGT GAT ACG GTT	thermonuclease	Brakstad *et al*. (1992)
Nuc R	AGC CAA GCC TTG ACG AAC TAA AGC	*ditto*	*ditto*
3 F	GCT TTT TAA GTG TAC TAT TC	catalase	this study[a]
164 F	TAT AAA TTG TGG AGG GAT GAC	*ditto*	Sanz *et al*. (2000)
8 F	CTC CAT TTT AGA ACG CAA CAA	*ditto*	*ditto*
1396 F	GAT GGA TAC GGC TAT GAA TA	*ditto*	this study[a]
872 R	GCT ATA ATT TCA GCA GCT TC	*ditto*	*ditto*
1583 R	TGG GTC AGC TTT GTA ACA	*ditto*	Sanz *et al*. (2000)
1726 R	TCA TAA ACT GCT CAA CTA CGC	*ditto*	*ditto*

[a]The primers were designed based on the sequences of the catalase genes of *Staphylococcus aureus* strain MVF213 (*GenBank* accession no. AJ000471) and *S. aureus* strain ATCC 12600 (*GenBank* accession no. AJ000472).

RESULTS AND DISCUSSION

Identification of the isolates by biochemical tests was confirmed by PCR amplification of the nuclease and catalase genes to the species level only (*i.e. S. aureus*) but not to the subspecies level. Further genetic characterization could be made by sequencing the catalase gene. Sequence of the putative catalase gene of *S. aureus* ssp. *anaerobius* strain S10 (SaanS10) showed 99 % identity to *katB* gene of *S. aureus* ssp. *anaerobius* MVF213 (*GenBank* accession no. AJ000471), *katA* gene of *S. aureus* ssp. *aureus* strains NCTC 8325 and Newman (*GenBank* accession nos CP000253 and AP009351.1, respectively) and some other strains. The whole amplified part of the putative catalase gene of SaanS10 (*katS10*) was 1725 nucleotides in length. Comparison of this sequence with *katB* sequence revealed mismatches of only three bases. Fifteen base substitutions occurred within the coding region, when compared with *katA*, six of which were missense mutations while the others were silent mutations. An important substitution occurred at position no. 1099

(1036 bases upstream of the initiation codon) of *katS10* gene. In *katS10* the base is "T", while in *katA* and *katB* it is "C". This substitution resulted in the code "TGA" instead of "CGA". This code for termination of translation rendered the predicted protein to be only 345 amino acids in length. In *S. aureus* ssp. *aureus* (NCTC 8325 and Newman strains) the protein of *katA* is 505 amino acids long. In *S. aureus* ssp. *anaerobius* strain MVF213, which is catalase negative, the catalase-like protein of *katB* is predicted to have 445 amino acids. The loss of the catalase activity of *S. aureus* ssp. *anaerobius* is attributed to deletion of one base 1338 nucleotides upstream to the initiation codon, which resulted in shift in the reading frame and premature termination of translation 30 bases later (Sanz *et al*. 2000). In *katS10* this deletion is absent, a feature of similarity to *katA*. The third mismatching of *katS10* and *katB* is that the substitution which occurred at base 949 upstream of the initiation codon leading to serine in *katB* instead of proline in *katA* (Sanz *et al*. 2000) did not happen in *katS10*. Interestingly, all mutations, except the above mentioned ones, occurred in *katA* leading to the generation of *katB* are similar to those mutations associated with *katS10*. This suggests that *katA* underwent mutations in at least two steps leading to the generation of *katB* and *katS10*.

To investigate for the presence of those mutations of *katB* and *katS10* in other *S. aureus* ssp. *anaerobius* isolates, partial sequences (lengths of ≈600 and ≈990 bp) of the catalase genes of 8 other isolates in addition to a reference strain (*S. aureus* ssp. *anaerobius* DSM 20714/ATCC 35844) were performed. The segment of the gene chosen for this partial sequence targeted a region that contained most of the mutations seen in *katB* and *katS10*, especially those at positions 1503 and 1099, respectively. The sequence of the catalase gene of all local isolates was identical to that of *katS10*, while that of the reference strain was identical to *katB* sequence.

S. aureus ssp. *anaerobius* strain MVF213 was originally isolated from a lamb suffering from abscess disease in Spain. The mutations found in this strain leading to the generation of *katB* were also found in three other strains isolated from lambs affected by the same disease in Spain in different years (Sanz *et al*. 2000). The Spanish strains thus seem to have originated from one clone (European clone), and the local strains harboring *katS10* seem to originate from another genetically distinct clone (African clone). This assumption can be augmented by the results of Elhaj and El Sanousi (2005) who found that local isolates of *S. aureus* ssp. *anaerobius* were identical, but distinct from the reference strain, in the DNA restriction pattern in PFGE.

In conclusion, this study shows clear distinction between local and reference strains of *S. aureus* ssp. *anaerobius* on the basis of the catalase gene sequence. Moreover, our results show that the catalase gene sequence can be used to describe development of regionally specific clones of *S. aureus* ssp. *anaerobius*. Use of the catalase gene as gene marker for typing strains of *S. aureus* ssp. *anaerobius* for epidemiological investigations could be further substantiated by studies that will utilize a greater array of isolates from various outbreaks in different geographic sites.

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