Characterization of a Novel Thermostable Nuclease Homolog (NucM) in a Highly Divergent Staphylococcus aureus Clade

Frieder Schaumburg, Maude Pauly, Grit Schubert, Adebayo Shittu, Steven Tong, Fabian Leendertz, Georg Peters, Karsten Becker

Institute of Medical Microbiology, University Hospital Münster, Münster, Germany; Research Group Emerging Zoonoses, Robert-Koch-Institut, Berlin, Germany; Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria; Menzies School of Health Research, Darwin, Northern Territory, Australia

A highly divergent Staphylococcus aureus clade in monkeys, bats, and great apes from Gabon, Nigeria, and Côte d’Ivoire was previously reported (1, 4). Besides showing species-specific characteristics of S. aureus (e.g., a typical 16S rRNA gene sequence), this clade was positive in a DNase agar test. However, no PCR amplicon was detected using species-specific primers targeting the S. aureus thermostable nuclease gene (nuc1), suggesting a modified nuc gene (3).

The objective of this study was to characterize the thermostable nuclease from this divergent S. aureus clade. The nuc PCR-negative isolates included in this study were derived from Gabon (n = 16), Nigeria (n = 2), Côte d’Ivoire (n = 11), and Democratic Republic of the Congo (n = 6) (see Table S1 in the supplemental material) (1, 2, 4, 5). These were identified as S. aureus isolates by (i) vitek 2 automated systems (bioMérieux, Marcy l’Étoile, France), (ii) 16S rRNA gene sequencing, and (iii) a positive tube coagulase test (6).

All the isolates were spa typed, and one isolate of each spa type was subjected to multilocus sequence typing (MLST) (7, 8). A neighbor-joining tree was constructed with MEGA 5.05 (www.megasoftware.net) using the concatenated MLST sequences of our isolates, major methicillin-susceptible S. aureus lineages in Africa (sequence type 5 [ST5], ST8, ST15, ST30, ST121, and ST152), a Staphylococcus simiae isolate (GenBank accession numbers FJ705815 to FJ705820), and ST1223 (9). The topology of the tree shows two divergent groups (see Fig. S1 in the supplemental material). The split into group 1 (reference STs) and group 2 (majority of nuc PCR-negative isolates) was supported by a bootstrap value of 100%.

We tested if the divergent (nuc PCR-negative) isolates produce a thermostable nuclease. The activity of the thermostable nuclease was spectrophotometrically measured in three independent experiments (10). Nuclease activities are expressed in absorbance units (AU) ± the standard deviation. In contrast to S. epidermidis ATCC 12228 (negative control), S. aureus ATCC 25923 showed positive control and isolates belonging to the divergent clade showed similar enzymatic activities (Fig. 1). This finding suggests that the divergent clade produces a thermostable nuclease, which has activity similar to that of the S. aureus nuc gene.

We therefore screened a set of primers targeting conserved flanking regions of the nuc gene in S. aureus (Mu50) and tested these primers on the divergent clade (see Table S2 in the supplemental material). The amplicons (749 bp) were sequenced (see Table S2); contigs were assembled and trimmed with Lasergene SeqMan Pro version 10.1.1 (DNASTAR, Madison, WI, USA). The GenBank accession numbers of the designated nucM sequences are KJ748637 through KJ748639.

A neighbor-joining tree was constructed using the nucM sequences and nuc sequences of S. aureus N315 (nuc1, SA0746; nuc2, SA1160), S. aureus Mu50 (SAV0815), S. aureus MW2 (MW1211), Staphylococcus carnosus TM300 (Sca_0025), Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305 (SSP1444), and Staphylococcus epidermidis ATCC 12228 (SE100) obtained from GenBank. Nuc1 of S. aureus standard strains (MW2, N315, Mu50) and nucM of the divergent clade were separated into two sister clades (0.160 base substitutions per site, assessed by the maximum composite likelihood method) (Fig. 2). The distance between nuc2 and nucM was even greater (1.64 base substitutions per site) than the distance between nuc1 and nucM. It is noteworthy that the nuc sequence of the isolates belonging to ST395 (n = 2) and ST1854 (n = 4) clustered with nucM of groups 1 and 3, respectively (Fig. 2; see also Table S1 in the supplemental material), although they were closely related to S. aureus reference strains based on concatenated MLST sequences (see Fig. S1 in the supplemental material). This might indicate a small intersection of classical S. aureus lineages and the divergent clade (see Fig. S1), or it might point toward a rare occurrence of the nucM homologue in classical S. aureus strains. Reports on nuc-negative S. aureus in the literature are rare. In one case, a 292-bp deletion at the S’ end of nuc in a methicillin-resistant S. aureus isolate was detected (11). Fosheim et al. reported two atypical S. aureus strains which were biochemically identified as S. aureus strains and showed a thermostable nuclease activity but were nuc PCR negative (12).

The evolutionary divergence within nucM was low, as expressed
by an average of 0.018 base substitutions per site. However, three subgroups of nucM were identified and associated with their geographical origins. Isolates from Côte d’Ivoire clustered in group 1, group 2 consisted of isolates from Nigeria and Gabon, and isolates from Gabon and the Democratic Republic of the Congo clustered in group 3 (Fig. 2). The mean distances of group 1 to group 2 and group 3 were 0.045 and 0.035 base substitutions per site, respectively. The mean nuclease activities of each nucM group were similar (data not shown).

The geographic clusters of nucM were also detected in the neighbor-joining tree using the concatenated MLST sequences (see Fig. S1 in the supplemental material). This suggests that the divergence of nucM parallels the divergence of the core genome of these isolates. Whole-genome sequencing is warranted to confirm this finding.

The amino acid sequences from the nuc gene of S. aureus N315 and from one isolate of each NucM subgroup (group 1, Cb1b; group 2, A096; group 3, FSA084) were deduced with ExPASy (http://web.expasy.org) (see Fig. S2 and Table S1 in the supplemental material). A comparison of the amino acid sequences of Nuc1 with NucM showed high identities and amino acid property similarities for Cb1b (80.4% identity, 94.1% similarity), A096 (78.1% identity, 93.3% similarity), and FSA084 (78.7% identity, 92.4% similarity) as computed by the FASTA sequence comparison tool (http://fasta.bioch.virginia.edu).

All deduced amino acid sequences had a signal peptide predicted by SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP) and contained the conserved active sites Arg114, Glu122, Arg166 (see Fig. S2 in the supplemental material). This may explain the similar nuclease activities.

**FIG 1** Nuclease activity and tertiary structure of Nuc. (a) The nuclease activities of all divergent S. aureus isolates (n = 35) were measured after incubation of the bacterial cells for 20 min at 100°C in three independent experiments. Error bars show the standard deviations, which are displayed in only one direction for the sake of clarity. The deduced amino acid sequences of S. aureus N315 and the divergent clade strain (FSA084) were used to predict the three-dimensional structures of NucM (b) and Nuc1 (c) with EsyPred3D. Cylinders represent alpha-helices, and arrows represent beta sheets.

**FIG 2** Phylogenetic relation of nuc sequences. Sequences of nuc genes from S. aureus (nuc1 and nuc2), the divergent clade, Staphylococcus saprophyticus subsp. saprophyticus, Staphylococcus carnosus, and Staphylococcus epidermidis were used to construct a neighbor-joining tree. Groups 1, 2, and 3 (nucM) were collapsed for the sake of clarity. Bootstrap scores of ≥75% are shown. The bar charts represent the proportions of isolates from Côte d’Ivoire, Nigeria, Gabon, and the Democratic Republic of the Congo. The countries of origin are shaded.
As the NucM amino acid sequences of the isolates belonging to the divergent clade were highly similar, we selected the dedicated type strain (FSA084) to compute the tertiary structure of NucM using ESyPred3D software (13). Comparison of the three-dimensional structures showed high homology of the thermostable nucleases from S. aureus N315 and the divergent isolate (FSA084) (Fig. 1). The predicted tertiary structure is similar to the crystal structure as assessed by crystallization consisting of a five-stranded beta-barrel and three alpha-helices (14).

In conclusion, the divergent S. aureus clade harbors a homologue of the thermostable nuclease (NucM), whose nucleotide sequence is highly divergent from those of nuc1 and nuc2 of S. aureus reference strains. This explains the lack of detection of nucM by standard nuc PCR approaches (3). The similar deduced amino-acid sequences and tertiary structures explain the similar nuclease activities.

ACKNOWLEDGMENTS

We are grateful for the support of the Albert Schweitzer Hospital and the Centre de Recherche Médicale de Lambaréné for our staphylococcal projects in Gabon.

This study was funded by the Deutsche Forschungsgemeinschaft (Infection Biology and Epidemiology of Staphylococci and Staphylococcal Diseases in Central and South Africa, grants PAK296, EL 247/8 and LE 1813/4-1).

REFERENCES


