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Short Communication

Distribution of the putative virulence factor encoding gene *sheta* in *Staphylococcus hyicus* strains of various origins

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In the present study, *Staphylococcus* (*S.*) *hyicus* strains isolated in Russia (n = 23) and Germany (n = 17) were investigated for the prevalence of the previously described genes *sheta* and *shetb*. *Sheta* was detected in 16 *S. hyicus* strains. *Sheta*-positive strains were mainly found among strains isolated from exudative epidermitis, and frequently together with the exfoliative toxin-encoding genes *exh*D and *exh*C. Partial sequencing of *sheta* in a single *S. hyicus* strain revealed an almost complete match with the *sheta* sequence obtained from GenBank. None of the *S. hyicus* strains displayed a positive reaction with the *shetb*-specific oligonucleotide primer used in the present study. According to the present results, the exotoxin encoding gene *sheta* seems to be distributed among *S. hyicus* strains in Russia and Germany. The toxigenic potential of this exotoxin, which does not have the classical structure of a staphylococcal exfoliative toxin, remains to be elucidated.

Keywords: exfoliative toxins, exudative epidermitis, *sheta, shetb, Staphylococcus hyicus*

Staphylococcus (*S.*) *hyicus* is a worldwide causative agent of exudative epidermitis in pigs, a generalized infection of the skin characterized by exudation, exfoliation, and vesicle formation [10]. *S. hyicus* isolated from exudative epidermitis generally produces exfoliation-inducing exotoxins, which show a close relation to comparable exfoliative toxins produced by *Staphylococcus aureus* isolated from Staphylococcal scaled skin syndrome infections in humans [7]. Exudative epidermitis-inducing exotoxins, originally described by Amtsberg [2], have been identified and purified from *S. hyicus* strains in Japan and Denmark and have been designated as SHETA and SHETB [8] and ExhA, ExhB, ExhC, and ExhD [1], respectively. The prevalence of the exfoliative toxin genes *exh*A, *exh*B, *exh*C, and *exh*D has been described for *S. hyicus* strains isolated from various countries [3-6]. However, little is known about the distribution of SHETA- and SHETB-encoding genes in *S. hyicus* or the combined occurrence of SHETA and SHETB and the exfoliative toxin-encoding genes described in Denmark. The present study was designed to investigate the distribution of *sheta* and *shetb* among previously characterized *S. hyicus* strains isolated in Russia and Germany.

A total of 40 *S. hyicus* strains, originally isolated in Russia and Germany, were investigated in the present study. The 40 *S. hyicus* strains and the reference strains *S. hyicus* S3588 (*exh*A), *S. hyicus* 1289D-88 (*exh*B), *S. hyicus* 842A-88 (*exh*C), *S. hyicus* A2869C (*exh*D), and *S. hyicus* DSM 20459 were identified and further characterized as described previously [6,9].

The *sheta* and *shetb* sequence data were obtained from GenBank (AB036768, AB036767). The design of the *sheta*- and *shetb*-specific oligonucleotide primers was performed using the computer program Oligo 4.0. The oligonucleotide primers used had the sequence 5`-GAACACGTTTTTCAGCCATATCTCC-3` and 5`-CGATTACAGTTGCCAATACCGTTTC-3` for *sheta* and 5`-GAGGCTTTACAGCCAAAATTATATGCTAG-3` and 5`-CAAATCGCTTCCTAGAGTATCTATTTTTTG-3` for *shetb*. Both oligonucleotide primers were synthesized by Operon (Germany).

The DNA preparation has been described previously [6]. The reaction mixture for *sheta* and *shetb* amplification contained 0.7 µl of each primer (10 pmol/µl), 0.8 µl of dNTP (10 mmol; Genecraft, Germany), 2.0 µl of 10 × Biotherm buffer with a final concentration of 1.5 mM $MgCl_2$ (Genecraft, Germany), 0.2 µl of Biotherm polymerase (Genecraft, Germany) and 13 µl of H_2O. The tubes were

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then subjected to thermal cycling (Gene Amp PCR System 2400; Perkin Elmer, Germany): 1 × 94°C for 180 sec; 30 × (94°C for 30 sec, 58°C for 30 sec, 72°C for 70 sec); and 1 × 72°C for 300 sec. The presence of PCR products was determined by electrophoresis of 10 µl of reaction product on a 1.5% agarose gel (Gibco BRL, Germany) with Tris-acetate electrophoresis buffer (TAE, 4.0 mmol/l Tris-HCl, 1 mmol/l EDTA, pH 8.0) and visualized under UV light (Image Master VDS; Pharmacia Biotech, Germany).

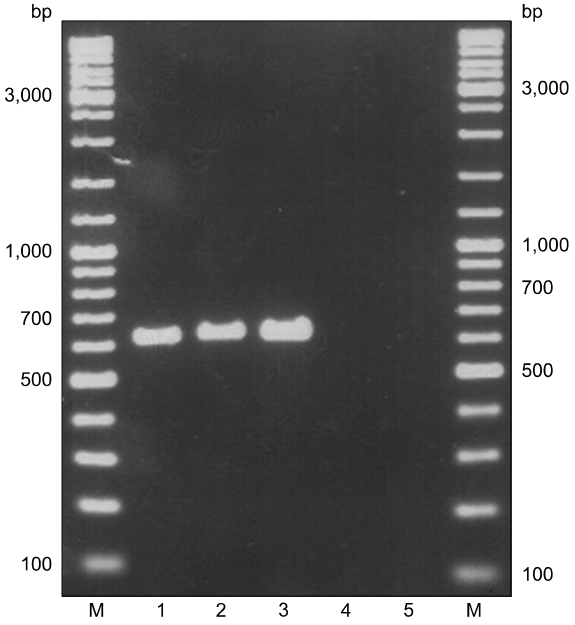

Fig. 1. Typical amplicons of *sheta*-positive *Staphylococcus hyicus* (1, 2, 3); *sheta*-negative Staphylococcus hyicus (4, 5).

For sequencing, the *sheta* amplicon of *S. hyicus* S3588 was eluted from the gel using QIAEX_II (Qiagen, Germany) according to the instructions of the manufacturer. The sequencing was performed using Sequence Genterprise (Mainz, Germany). A sequence comparison was carried out using the database of the National Centre for Biotechnology Information (NIH, USA). The toxin gene and protein sequences were compared with the exfoliative toxin gene and protein sequences of GenBank using a computer program, ClustalW (EBI, UK).

The *S. hyicus* strains investigated in the present study had been previously characterized and identified by phenotypic methods and by PCR-mediated amplification of *S. hyicus*-specific segments of the superoxide dismutase A-encoding gene *sod*A and by amplification of specific segments of the 16S-23S rDNA intergenic spacer region [9]. Screening of these strains for the exfoliative toxin genes *exh*A, *exh*B, *exh*C, and *exh*D by multiplex PCR revealed the presence of *exh*D in 17 of the *S. hyicus* strains isolated in Russia and the genes *exh*C and *exh*D for one and two *S. hyicus* strains, respectively, isolated in Germany [6]. Investigation of the *S. hyicus* strains for *sheta* and *shetb* yielded *sheta* positivity in 11 strains isolated in Russia and 5 strains isolated in Germany (Fig. 1). The origin of the strains and the PCR results are summarized in Table 1. Sequencing of the *sheta* gene from strain *S. hyicus* S3588 in the present study revealed an almost complete sequence match with the *sheta* sequence of GenBank. The sequencing results together with other available exfoliative toxin gene sequences are shown in Fig. 2. The presence of *sheta* in the present study occurred more frequently among strains isolated from exudative epidermitis, partly together with *exh*D and *exh*C. However, some of the *sheta*-positive strains were negative for *exh*C and *exh*D (Table 1). It was

Table 1. Toxigenic properties of 23 *Staphylococcus hyicus* strains isolated in Russia and 17 *Staphylococcus hyicus* strains isolated in Germany

Origin of the strain	Animal origin pig (n = 36), cow (n = 2), dog (n = 1), chicken (n = 1)	*sheta*-positive and *exh*C-positive	*sheta*-positive and *exh*D-positive	*sheta*-positive and *exh*C-, *exh*D-negative
Russia	Exudative epidermitis (pig, n = 19)	-	7	1
	Pneumonia (pig, n = 1)	-	-	-
	Without clinical symptoms (pig, n=2; chicken, n = 1)	-	2	1
Germany	Exudative epidermitis (pig, n = 7)	1	-	2
	Clinical symptoms with unclear relation to the isolation of *S. hyicus* (pig, n = 6; cow, n = 2; dog, n = 1)	-	1	-
	Without clinical symptoms (pig, n = 1)	-	-	1

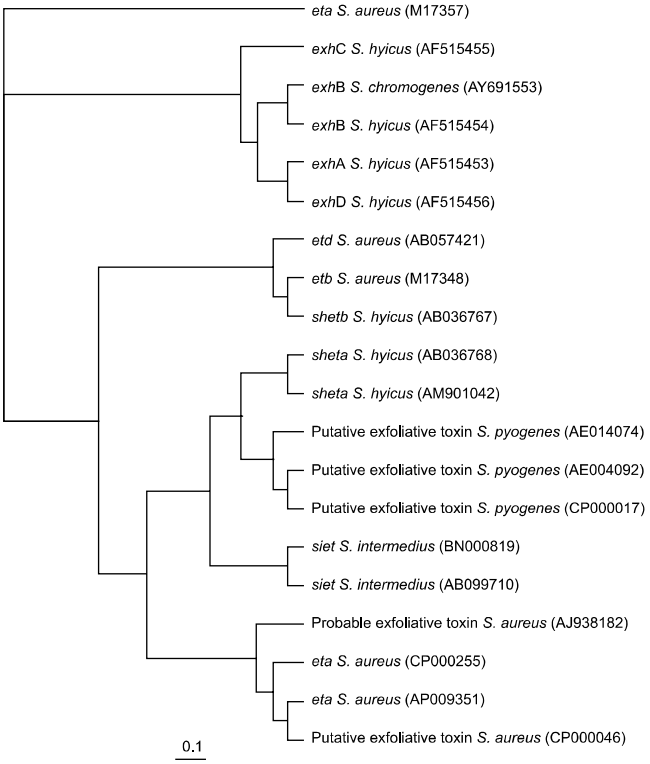

Fig. 2. Dendrogram analysis of the *sheta* gene sequence of the present study and the published exfoliative toxin gene sequences of the genera *Staphylococcus* and *Streptococcus*. The dendrogram was prepared using the computer program ClustalW.

interesting to note that the *exh*A, *exh*B, *exh*C, and *exh*D reference strains and *S. hyicus* strain DSM 20459, which was also isolated from exudative epidermitis and was described as *exh*A-positive, were also *sheta*-positive.

None of the strains was positive for *shetb*. The latter finding corresponded to the findings of Futagawa-Saito *et al.* [5]. These authors investigated 161 *S. hyicus* strains isolated from pigs with exudative epidermitis and 46 strains isolated from healthy pigs in Japan and could not detect plasmid-borne *shetb*. However, since no *shetb* reference strain is internationally available, the role SHETB plays in exudative epidermitis remains unclear.

According to Sato *et al.* [8] SHETA is under chromosomal control, and SHETB is plasmid-controlled. Strains investigated by Sato *et al.* [8] produced either SHETA or SHETB, or both. The typical signs of exudative epidermitis were observed in piglets inoculated with plasmid-carrying, SHETB-producing strains, as well as those inoculated with plasmidless SHETA-producing strains, indicating that both toxins seem to be involved in the clinical signs of exudative epidermitis. According to Ahrens and Andresen [1] and Yamaguchi *et al.* [11], SHETA does not posses the catalytic triad His-115, Asp-164, and Ser-239 of the S2 family of serine proteases, which is typical for the exfoliative toxins of S. aureus and *S. hyicus*, and also for SHETB. However, according to the findings of Sato *et al.* [8] and the results of the present study, SHETA seems to contribute to the clinical signs of exudative epidermitis, and PCR-amplification of *sheta* could additionally be used to detect virulent *S. hyicus*. At present, a target molecule or mode of action for SHETA has not been suggested.

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