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Comparative genomics of toxigenic and non-toxigenic *Staphylococcus hyicus*

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ABSTRACT

The most common causative agent of exudative epidermitis (EE) in pigs is *Staphylococcus hyicus*. *S. hyicus* can be grouped into toxigenic and non-toxigenic strains based on their ability to cause EE in pigs and specific virulence genes have been identified. A genome wide comparison between non-toxigenic and toxigenic strains has never been performed. In this study, we sequenced eleven toxigenic and six non-toxigenic *S. hyicus* strains and performed comparative genomic and phylogenetic analysis. Our analyses revealed two genomic regions encoding genes that were predominantly found in toxigenic strains and are predicted to encode for virulence determinants for EE. All toxigenic strains encoded for one of the exfoliative toxins ExhA, ExhB, ExhC, or ExhD. In addition, one of these regions encoded for an ADP-ribosyltransferase (EDIN, epidermal cell differentiation inhibitor) and a novel putative RNase toxin (polymorphic toxin) and was associated with the gene encoding ExhA. A clear differentiation between toxigenic and non-toxigenic strains based on genomic and phylogenetic analyses was not apparent. The results of this study support the observation that exfoliative toxins of *S. hyicus* and *S. aureus* are located on genetic elements such as pathogenicity islands, phages, prophages and plasmids.

1. Introduction

Staphylococcus hyicus is a gram-positive bacterium that can cause exudative epidermitis (EE) in pigs, and primarily piglets (Foster, 2012). EE is characterized by exfoliation of the skin and the formation of a thick, greasy, brown exudate (Jones, 1956) that may lead to dehydration and subsequent animal death (L'Ecuyer and Jericho, 1966). *S. hyicus* has been divided into pathogenic and non-pathogenic strains according to their ability to induce EE in pigs (Tanabe et al., 1996; Wegener et al., 1993) and their ability to produce exfoliative toxin which is the main virulence factor necessary to induce the disease (Andresen and Ahrens, 2004; Sato et al., 2000). Five exfoliative toxins from *S. hyicus* have been described so far, of which one has been characterized in Japan as SHETB (Sato et al., 2000) and four in Denmark as ExhA, ExhB, ExhC, ExhD (Andresen and Ahrens, 2004). The Exh toxins have been shown to cause loss of cell adhesion in the epidermis of porcine skin by cleaving desmoglein-1, while, human desmoglein-1 is resistant to *S. hyicus* exfoliative toxins (Fudaba et al., 2005; Nishifuji et al., 2005).

A genomic analysis and comparison between toxigenic and non-toxigenic strains potentially identifying other virulence markers has so far not been performed, and is the subject of the present study. A *S. hyicus* reference strain was recently completely sequenced (Calcutt et al., 2015). The present study was conducted to study the phylogeny of 17 different *S. hyicus* strains, and compare the genomes of toxigenic and non-toxigenic strains, and to identify potential additional virulence determinants.

2. Materials and methods

2.1. Bacterial isolates

The eleven toxigenic and six non-toxigenic *S. hyicus* strains investigated in this study have been previously characterized by their ability to cause generalized EE after inoculation in healthy 14-day-old piglets (Wegener et al., 1993) or their ability to produce exfoliative toxin (Andresen and Ahrens, 2004). The strains were from Denmark ($n=9$), Germany ($n=5$) and UK ($n=3$).

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2.2. Whole genome sequencing

Genomic DNA was extracted from the 17 *S. hyicus* strains using an Invitrogen Easy-DNA™ Kit (Invitrogen, Carlsbad, CA, USA) and DNA concentrations were determined using the Qubit dsDNA BR assay kit (Invitrogen). The genomic DNA was prepared for Illumina pair-end sequencing using the Illumina (Illumina, Inc., San Diego, CA) NexteraXT® Guide 150319425031942 following the protocol revision C (http://support.illumina.com/downloads/nextera_xt_sample_preparation_guide_15031942.html). A sample of the

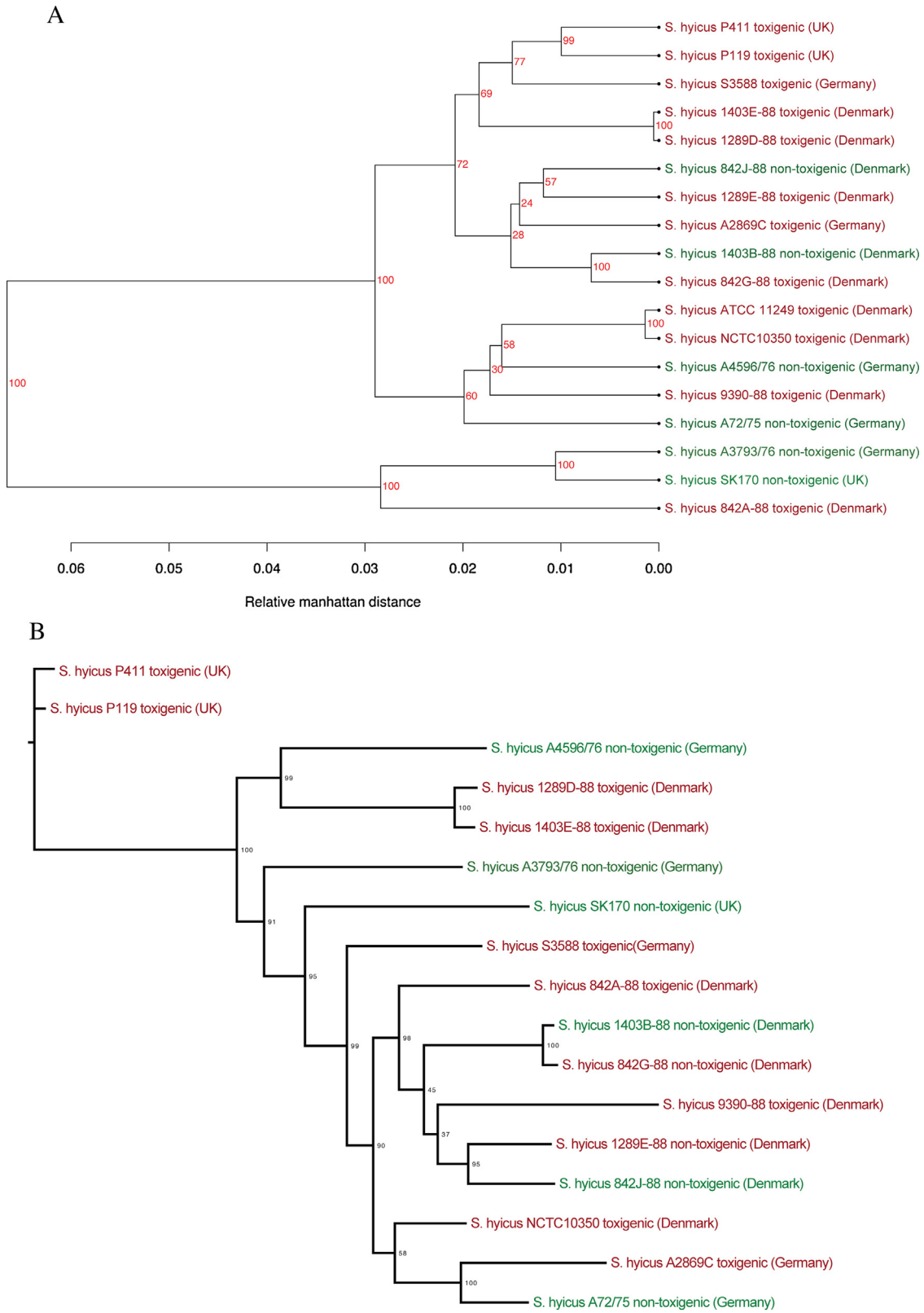

Fig. 1. Pan-genome tree (A) and SNP tree (B) of non-toxigenic (green) and toxigenic (red) *S. hyicus* strains. The numbers shown in branches are bootstrap values.