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Short communication

Detection of *Jeotgalicoccus* spp. in poultry house air

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ABSTRACT

Investigations of bioaerosols collected from turkey, chicken and duck houses, as well as from a duck slaughterhouse, each in triplicate, revealed that 4–18% of 16S rRNA gene sequences in investigated 16S rRNA gene clone libraries were closely related to *Jeotgalicoccus* spp. *J. halotolerans*- and *J. psychrophilus*-related sequences were obtained in all investigated bioaerosol samples and formed a distinct group with sequences of both species type strains, which were collectively entitled Jeot-cluster-I. For a quantification of Jeot-cluster-I bacteria, a group specific PCR primer combination targeting the 16S rRNA genes was developed. Estimated concentrations by quantitative real-time PCR analyses revealed cell numbers between 10^4 and 10^6 *Jeotgalicoccus* cells m^{-3} air in turkey, duck, and chicken houses, respectively. These results indicated the remarkable proportion (1–39%) of total cell counts and the hitherto unknown wide distribution of *Jeotgalicoccus* spp. in the poultry rearing industry.

The concentrations of airborne bacteria in poultry houses vary between 10^4 and 10^8 colony forming units (CFU) and 10^7 and 10^{10} cells m^{-3} air [16,20,24], respectively. Therefore, these plants are important microorganism emission sources (e.g. [2,25]). The exposure of humans and animals, as well as transmission into the environment, has led to an increasing interest in such bioaerosols during recent decades, in particular among local residents in the surroundings of these plants.

Generally, staphylococci have been described as predominant bacteria in agricultural environments [9,20,26], especially in poultry houses [21]. However, despite the fact that remarkable new taxa were detected [11–14,19] these airborne microbial communities are rarely investigated in detail. Recently, we observed *Jeotgalicoccus*, an additional Gram-positive, non-motile, coccus shaped genus of the family *Staphylococcaceae*, in bioaerosols (submitted). Consequently, we studied the abundance of *Jeotgalicoccus* in poultry houses in more detail to gain an insight into the composition of *Staphylococcaceae*, in particular the abundance of *Jeotgalicoccus*.

The genus *Jeotgalicoccus* is comprised, at the time of writing, of five species: *Jeotgalicoccus halotolerans*, *J. psychrophilus*, *J. marinus*, *J. pinnipedialis*, and *J. huakuii* [4,8,10,27]. *J. psychrophilus* and *J. halotolerans* were first isolated from traditional Korean fermented seafood, called "jeotgal", a few years ago [27]. Oppong et al. [22] recently showed that one species most closely related to *J. psychrophilus* was a bacterial contaminant of fresh hides, and Callon et al. [3] detected both species during bacterial community analyses of goat milk. Recently, *J. halotolerans* has been isolated during analysis of a microbial community in dark fire-cured tobacco fermentation [5]. *J. pinnipedialis* species were first described by Hoyles et al. [10] from a southern elephant seal.

To estimate the abundance and diversity of *Jeotgalicoccus* in poultry house bioaerosols, first we used 16S rRNA cloning approaches. For this, extracted DNA from a selection of nine bioaerosol samples of turkey houses, duck houses and duck slaughterhouses (three independent samples from each location), which were collected and processed in a previous study [6], were investigated. The PCR products obtained by the universal bacterial 16S rRNA gene primers [15] were cloned into *E. coli* using the pGEM®T Vector System Kit (Promega Corp., USA), according to the manufacturer's instructions. A total of 347 clones were randomly picked. Cloning and sequence analyses were carried out by Agowa (Berlin, Germany). Phylogenetic analyses of sequences were conducted as described earlier [18].

These investigations revealed that 4–18% of sequences were most closely related to the genus *Jeotgalicoccus* (Table 1). Sequences most closely related to *J. pinnipedialis* were detected only in bioaerosol samples of the investigated duck slaughterhouse (Fig. 1). *J. halotolerans*- and *J. psychrophilus*-related sequences were obtained in all investigated bioaerosol samples. These formed a distinct group with sequences of both type strain species, which we collectively called the Jeot-cluster-I (Fig. 1).

Due to the high prevalence of *J. psychrophilus*- and *J. halotolerans*-like sequences in all investigated samples, a Jeot-cluster-I specific primer combination (JeotF/JeotR) was designed

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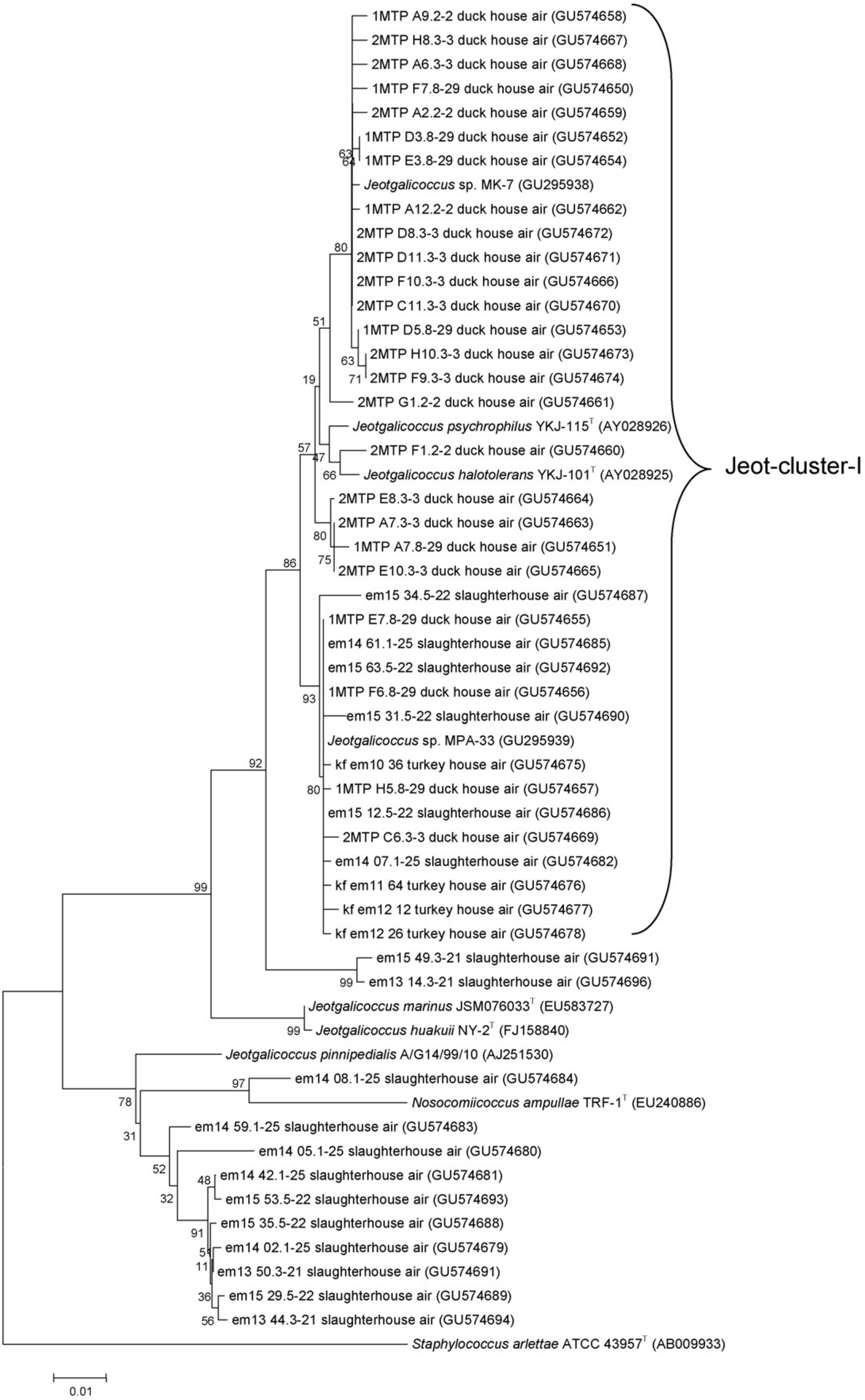

Fig. 1. Phylogenetic tree showing the position of *Jeotgalicoccus*-related 16S rRNA gene sequences (∼720 bp) derived from 16S rRNA gene clone libraries from bioaerosols of turkey house air, duck house air and duck slaughterhouse air (GU574650-GU574696). For each clone library, 64 clone inserts were analysed. The tree was constructed by the neighbour-joining method and calculated with the Kimura 2-parameter model. The numbers at the nodes indicate the percentage recovery in 1000 bootstrap resamplings. The scale bar corresponds to a 1% estimated difference in the nucleotide sequence position. Accession numbers are given in brackets.