Extension of multiplex PCR for *Streptococcus suis*

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Introduction

Streptococcus suis is an important zoonotic pathogen of swine. 29 different capsular serotypes have been described and novel *cps* loci are constantly emerging [1-5]. To distinguish between serotypes, Multiplex PCR tests have recently been developed that target specific genes in the *cps* loci of *S. suis* [6]. Here, we have developed an extension of the multiplex PCR method from Kerdsin et al. for non-serotypeable strains collected in the Czech Republic.

Materials and Methods

After Whole genome sequencing of *S. suis* isolates collected in the Czech Republic and analyzing the *cps* loci of non-serotypeable isolates, we designed 19 pairs of specific primers targeting unique sequences to detect potentially novel *cps* loci. After verifying the functionality of Singleplex PCR reactions, we developed three Multiplex PCRs to detect multiple isolates simultaneously.

Results

Initially, we successfully tested all 19 PCR reactions. We developed Multiplex PCR schemes for 15 primers, while the remaining 4 primers, with similar PCR product lengths, have not yet been included in the Multiplex PCR. We successfully tested two Multiplex PCRs, each capable of distinguishing five types. Despite successful Singleplex PCRs, our attempt to

verify the functionality of five additional primer pairs in the third Multiplex PCR was unsuccessful, indicating the need for optimization of the reaction conditions.

Discussion and Conclusion

We successfully developed two Multiplex PCRs, which can distinguish a total of 10 possible strains in two reactions. Although we were unable to design a third Multiplex PCR for the simultaneous differentiation of five strains, we believe that optimizing the conditions will allow us to achieve this. The extended Multiplex PCR allows for the identification of additional serotypes, thus reducing the number of untypeable *S. suis* strains.

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References

1 Okura M, et al. 2016. doi:10.3390/pathogens5030045

2 Zheng H, et al. 2015. doi.org/10.1128/AEM.00315-15

3 Qiu X, et al. 2016. doi.org/10.1128/AEM.02102-16

4 Zheng H, et al. 2017. doi.org/10.1186/s13567-017-0417-6

5 Huang J, et al. 2019. doi.org/10.1111/tbed.13123

6 Kerdsin A, et al. 2014. doi:10.1099/jmm.0.069757-0