
Poster: free communications

Development of an *in vitro* cultivation system for the haemotrophic bacterium *Mycoplasma suis*

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Mycoplasma suis, the causative agent of infectious anaemia in pigs (IAP), belongs to the group of haemotrophic mycoplasmas (haemoplasmas, HM). They colonize erythrocytes of a wide range of domestic and wild animals causing acute haemolytic anaemia or chronic anaemia with immune suppression. Hitherto, HM are regarded as uncultivable *in vitro*. Thus, research still relies on the propagation of HM in splenectomized animals, a method connected with serious ethical concerns. In regard to replace these animal experiments, this research project is aimed to establish an *in vitro* cultivation system by using *M. suis* as a model organism for all HM. It is based on the hypothesis that HM can be grown in classical *Mycoplasma*-specific media supplemented with appropriate nutrients as they are available in their natural environment, i.e. the mammalian blood. In our approach, all different culture systems are inoculated with anti-coagulated blood from experimentally infected pigs. The bacterial load of the *M. suis* cultures is periodically controlled and quantified by a *M. suis*-specific quantitative real-time PCR assay. Subcultivation is carried out in both liquid and softagar media and on *Mycoplasma*-standardized agar plates.

After the first cultivation period, a successful *in vitro* maintenance of *M. suis* has already been achieved, the most important step towards continuous growth. Moreover, after a long incubation time remarkable star-like shaped colony structures became visible on agar plates growing into the agar surface. In a next step, the switch to successful subcultivation in liquid media to get bigger quantities of *M. suis* will be our focus. In conclusion, these findings demonstrate for the first time that investigation and optimization of the *in vitro* requirements of *M. suis* growth could lead to the continuous *in vitro* propagation of *M. suis* and subsequent establishment of an appropriate culture system for all HM.

Keywords: *Mycoplasma suis*, haemoplasmas, *in vitro* cultivation, IAP, pigs