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Cytotoxicity of *Haemophilus somnus* Grown in Continuous Culture

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Abstract

Haemophilus somnus (*Histophilus somni*) is the cause of many disease manifestations in cattle and sheep including thrombotic meningoencephalitis (TME), pneumonia and reproductive disease. The effect of temperature and pH on the growth kinetics of *Haemophilus somnus* in continuous culture were investigated in a previous study. In this study, we present primary results of the investigation of the effects of temperature and pH on the cytotoxic activity of *Haemophilus somnus*. *Haemophilus somnus* 8025 (type strain) was grown in continuous culture system using the bioreactor (fermenter) of the Institute of Biotechnology in the Tropics (IBT), Göttingen, at each pH for 24 hours and at each temperature for 12 hours. The culture temperature was set at 37°C for studying the pH effect; for the temperature the pH was set at 7.1, while the culture agitation and dilution rates were kept constant. An MTT assay was used to determine the cytotoxicity of the cell free culture filtrate (CFCF) to MDCK cells. Fermenter cultures at different temperatures (29, 31, 33, 35, 37, 39 and 41°C) and pH (6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8 and 8.2) were tested. CFCF was 2-fold serially diluted in 96 well tissue culture plates. Non filtered Supernatant of some cultures was also tested for comparison. Controls of MDCK Cells, trypsin and fermenter medium were included in each plate. CFCF and supernatant of cultures at all pH and all temperatures tested, except at 29°C, were Cytotoxic to MDCK cells at the lower dilutions.

Keywords: Continuous culture, *Haemophilus somnus*, MTT assay