Optimization of in vitro gowth conditions and DNA extraction from *Treponema phagedenis* isolated from bovine digital dermatitis lesions

A. Krull, *DVM*; **P. Plummer**, *DVM, PhD, DACVIM (LA)*
Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011

Introduction

Bovine digital dermatitis is a leading cause of lameness in the US dairy industry, and it also represents a significant welfare issue. Our group and others have demonstrated that, along with a number of other organisms, *Treponema* spp can be cultured from a large percentage of digital dermatitis lesions. As part of our ongoing effort to better understand the role that *Treponema* spp plays in this disease process, we are using a combination of culture-dependent and independent methods to better understand the physiology and metabolism of these bacteria. *Treponema* spp are fastidious and their growth in broth media has traditionally been difficult; thus; hindering the ability to grow sufficient organisms for DNA purification to be used in downstream sequencing or genetic modification. This study details the systematic evaluation of different culture conditions on the growth and replication of *T. phagedenis* isolated from digital dermatitis lesions of cattle.

Materials and Methods

The impact of oxygen concentration (anaerobic, microaerophilic, and aerobic), inclusion of fetal calf serum (5, 10, or 15%), shaking, and incubation temperature were tested in broth culture of the organism. Three *T. phagedenis* isolates obtained from digital dermatitits lesions were grown under varying conditions for six days with daily OD readings as well as quantification and analysis using flow cytometry and Live/Dead staining. At log phase growth, DNA extraction was completed by use of several commercially available kits, along with modifications recommended from results of research involving other *Treponema* species.

Results

Under optimal growth conditions, broth cultures reached greater than $1.0x10^8$ bacteria per mL of broth in 96 hours. Although total bacteria numbers and optical density continued to rise past 96 hours, the total number of live cells decreased past 96 hours. Therefore, log phase should be considered 72-96 hours, followed by a short stationary phase, with cells entering the death phase by 120 hours. Extraction of DNA from these cultures was optimized with respect to commercially available kits, amount of starting material, and optional Proteinase K and RNase options. As a result of this optimization, cultures consistently yielded > 20ug of high quality DNA.

Significance

The results indicate that modifications to the traditional culture techniques employed for *T. phagedenis* result in enhanced growth and that use of these techniques results in large quantities of high-quality DNA in < 4 days. These findings lay the foundation for an improved ability to cultivate this *T. phagedenis* for use in challenge experiments and for the development of improved tools for genetic manipulation of *Treponema* spp.