Generation of *Histophilus somni* Mutants Deficient in Biofilm Formation or Attenuated in Virulence

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Introduction

Histophilus somni is an etiologic agent of bovine respiratory disease and various systemic diseases. A variety of virulence factors have been identified in *H. somni*, such as the lipooligosaccharide phase variation, the filamentous hemagglutinin, biofilm formation, and others, which act primarily to protect the pathogen against host defense mechanisms. Thorough investigation of the role of these virulence factors has been compromised because mutagenesis of *H. somni* using allelic exchange is difficult due to the apparently tight restriction modification system in virulent strains.

Materials and Methods

We employed the commercial Tn5 transposon EZ::Tn5TM Tnp Transposome (Epicentre) to generate a bank of mutants, and a random amplification of transposon ends (RATE) sequencing method to identify the transposon insertion sites. Mutants were screened for biofilm formation by binding of crystal violet to cultures grown in microtiter plates or tubes. Mutant isolates were tested for virulence in a Swiss-Webster model of septicemia and systemic disease.

Results

Screening of the mutant bank for isolates altered in biofilm formation resulted in the identification biofilm-deficient isolates with mutations in fhaC (outer membrane transporter protein for secretion of the filamentous hemagglutinin), uspE (a universal stress protein essential for cell motility and aggregation), and tolC (encoding the type I secretion outer membrane protein). Furthermore, a random screening of mutants with normal biofilm formation identified an isolate with a mutation in luxS, which is responsible for production of the autoinducer (AI) of the AI-2 quorum sensing pathway that is present in most gram-negative bacteria. luxS and quorum sensing have been shown to be important in biofilm formation in some bacteria, such as *Pseudomonas aeruginosa*, and virulence in others, such as *Haemophilus influenzae* and *Vibrio cholerae*. In a Swiss Webster mouse model of septicemia and systemic disease, the uspE and luxS mutants were highly attenuated, causing no disease or clinical symptoms at a challenge dose of >2.5 X 10^8 colony forming units [CFU]) (LD50 of the parent is < 5 x 10^7 CFU). Of interest though, was following the first immunization with the luxS mutant, most mice (9 of 15) went into shock and died on the same day following a second immunization with the mutant.

Significance

Additional strains of mice and cattle are being tested to determine if the luxS and/or uspE mutants are capable of inducing protection against challenge with virulent strains. Transposon mutagenesis with EZ::Tn5TM Tnp Transposome is an efficient method for generating mutants of *H. somni* to identify virulence factors and their role in bovine respiratory disease.