

**Cloning and Characterization of a Gene Involved in  
Lipooligosaccharide  
Biosynthesis in *Haemophilus somnus***

Jennifer A. Hensley

Thesis submitted to the Faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of

Master of Science  
Veterinary Medical Sciences

Dr. Thomas J. Inzana, Chair

Dr. Stephen M. Boyle

Dr. Brenda Winkel Shirley

Defense Date: May 6, 1998

Blacksburg, VA

Keywords: *Haemophilus somnus*, lipooligosaccharide, phase variation, CAAT repeats

## Abstract

### Cloning and Characterization of a Gene Involved in Lipooligosaccharide Biosynthesis in *Haemophilus somnus*

Jennifer A. Hensley

Committee Chairman: Thomas Inzana  
Veterinary Medical Sciences

Repetitive tetramers of the DNA sequence 5'-CAAT-3' are present in several loci associated with lipooligosaccharide (LOS) phase variation in *Haemophilus influenzae* type b (Hib). In an attempt to identify *H. somnus* phase-variable LOS genes, the presence of CAAT repeats within the *H. somnus* 738 genome was confirmed using a (CAAT)<sub>7</sub> probe. A 3.9 kb *EcoRI* fragment that reacted with the probe was cloned and sequenced. Sequence analysis confirmed the presence of 31 CAAT repeats downstream of two potential start codons, and indicated that small or large proteins would be encoded depending on the number of CAAT repeats. The larger gene products showed 46% amino acid homology to Lex2b from Hib, which influences LOS phase variation in that species. In *H. somnus*, this gene was named *lob1* (lipooligosaccharide biosynthesis gene). Sequence analysis showed that randomly selected colonies most frequently contained 33 CAAT repeats in *lob1*, corresponding to a 294 amino acid product. Colonies selected for negative reactivity to mAb 5F5 were significantly more likely to have different numbers of CAAT repeats in *lob1* than randomly selected colonies. The presence of *lob1 in trans* altered the LOS profile of a non-phase variable strain of *H. somnus*, and caused increased levels of reactivity to polyclonal antisera made to purified LOS from strain 738. Based on the ability of this gene to alter the LOS profile of a non-phase varying strain and the correlation of changes in CAAT repeats with mAb 5F5 reactivity, *lob1* appears to be involved in LOS biosynthesis and phase variation.

## **Acknowledgments**

Many people deserve credit for the research presented in this thesis. I thank my advisor, Dr. Tom Inzana, for providing me with the opportunity to work on this project and for teaching me that “good” research requires expertise in many areas. I thank my committee members, Dr. Stephen Boyle and Dr. Brenda Shirley, for providing excellent guidance and support. I thank current and former members of my lab, especially Gretchen Glindemann, Maureen Fallon, Christine Ward, Yanping Wu, Mark Lawrence, and Mike Howard, as well as friends like Simge Bolaglu, for providing technical help and laughter in all aspects of my work. I thank Dr. Eyre, Dr. Lee, and Linda Price, without whose financial and administrative support I could not have even dreamed of completing a DVM and a Master’s degree in only five years.

To my parents and extended family, thank you for supporting my dreams and for encouraging me to always achieve more than I thought I could. Most especially, I thank my fiancé, John McQuiston, who never hesitated to provide help or advice, even over the breakfast table. He was an indispensable part of this project, and I could not ask for a better friend.

This research was conducted as part of the Parallel Program in Veterinary Medicine at Virginia-Maryland Regional College of Veterinary Medicine, and was supported by the college’s Office of Research and Graduate Studies.

## Table of Contents

Abstract.....	p. ii
Acknowledgments.....	p. iii
List of Tables.....	p. v
List of Figures.....	p. vi
List of Abbreviations.....	p. vii
Chapter 1: LITERATURE REVIEW.....	p. 1
Chapter 2: RATIONALE FOR THESIS.....	p. 9
Chapter 3: MATERIALS AND METHODS.....	p. 10
Bacterial Strains and Growth Conditions.....	p. 10
Enzymes and Reagents.....	p. 10
Genomic DNA Extraction.....	p. 12
Plasmids.....	p. 12
DNA Hybridization Studies Confirming CAAT Repeats.....	p. 12
Cloning of the <i>Eco</i> RI Fragment Containing CAAT Repeats.....	p. 15
DNA Sequence Analysis of pCAAT-1 and pCAAT-2.....	p. 15
Colony Immunoblots.....	p. 15
PCR Amplification and Sequence Analysis.....	p. 16
Statistical Analysis.....	p. 17
LOS Extraction and Analysis.....	p. 17
Studies of the Effects of <i>lob1 in trans</i> .....	p. 17
Mutagenesis of <i>lob1</i> Using Allelic Exchange.....	p. 19
Detecting the Presence of <i>lob1</i> in Other <i>H. somnus</i> Strains.....	p. 20
Chapter 4: RESULTS.....	p. 22
Confirmation of CAAT Repeats in <i>H. somnus</i> Strain 738.....	p. 22
Correlation of CAAT Repeats to Phase Variation.....	p. 27
Effects of <i>lob1 in trans</i> .....	p. 30
Attempted Mutagenesis of <i>lob1</i> Using Allelic Exchange.....	p. 33
Presence of <i>lob1</i> in Other <i>H. somnus</i> Strains.....	p. 36
Chapter 5: DISCUSSION.....	p. 40
REFERENCES.....	p. 48
APPENDIX A.....	p. 55
Curriculum Vitae.....	p. 59

## List of Tables

Table 1.1: Loci with tetrameric repeats in <i>H. influenzae</i> type b.....	p. 7
Table 3.1: Bacterial strains used in this research.....	p. 11
Table 3.2: Plasmids used in this research.....	p. 13

## List of Figures

Figure 4.1: Southern hybridization analysis of genomic DNA.....	p. 23
Figure 4.2: Restriction map of the 3.9 kb <i>EcoRI</i> fragment from pCAAT.....	p. 24
Figure 4.3: DNA sequence of <i>lob1</i> .....	p. 25
Figure 4.4: Amino acid homology between Lob1 and Lex2b.....	p. 26
Figure 4.5: Reactivity of <i>H. somnus</i> strain 738 to mAb 5G8.....	p. 28
Figure 4.6: Reactivity of <i>H. somnus</i> strain 738 to mAb 5F5 .....	p. 29
Figure 4.7: Electrophoretic profiles of LOS from <i>H. somnus</i> strain 738 isolates	p. 31
Figure 4.8: Effects of <i>lob1 in trans</i> on <i>H. somnus</i> strain 129Pt phenotype.....	p. 32
Figure 4.9: SDS-PAGE of LOS from serially passed isolates of <i>H. somnus</i> .....	p. 34
Figure 4.10: <i>lob1</i> mutant construct for allelic exchange.....	p. 35
Figure 4.11: Detection of <i>lob1</i> in other <i>H. somnus</i> strains.....	p. 37
Figure 4.12: SDS-PAGE LOS profiles of <i>H. somnus</i> 129Pt.....	p. 38
Figure 5.1: Elucidated structure of <i>H. somnus</i> strain 738 LOS.....	p. 45
Figure 5.2: Arrangement of <i>lob</i> genes in <i>H. somnus</i> strain 738.....	p. 47

## Abbreviations

aa = amino acid  
Amp = Ampicillin  
BHI = Brain Heart Infusion  
bp = basepair  
C = Celsius  
CBA = Columbia Blood Agar  
dig = digoxigenin  
DNA = deoxyribonucleic acid  
dNTP's = deoxynucleotide triphosphates  
Gal = galactose  
Glc = glucose  
GlcNAc = glucose-N-acetylglucosamine  
GalE = UDP-4-galactose epimerase  
Hep = heptose  
Hib = *Haemophilus influenzae* type b  
HPLC water = high performance liquid chromatography water  
HRP = horse radish peroxidase  
kb = kilobase  
kDa = kilodalton  
Km = kanamycin  
KmR = kanamycin resistant  
kv/cm = kilovolt per centimeter  
KDO = 3-deoxy-D-manno-2-octulosonic acid  
LB = Luria Broth  
LOS = lipooligosaccharide  
LPS = lipopolysaccharide  
mAb = monoclonal antibody  
M = molar  
MCS = multiple cloning site  
mM = millimolar  
NAD = nicotinamide adenine dinucleotide  
nmol = nanomole  
OD = optical density  
ORF = open reading frame  
P = phosphate  
PAGE = polyacrylamide gel electrophoresis  
PC = phosphorylcholine  
PCR = Polymerase Chain Reaction  
PE = phosphoethanolamine  
rpm = revolutions per minute  
SDS = Sodium dodecyl sulfate  
SSC = Sodium chloride / Sodium citrate Buffer (0.15M NaCl, 0.015M Sodium citrate)  
Strep = Streptomycin  
TBS = Tris-buffered Saline (146mM NaCl, 10mM Tris-Cl, pH 7.4)  
TE = Tris-EDTA Buffer (10mM Tris-Cl, 1mM EDTA, pH 7.5)  
TME = thrombotic meningoencephalitis  
TMP = thiamine monophosphate  
ug = microgram  
ul = microliter  
UV = ultraviolet