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

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Abstract

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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 $(CAAT)_7$ probe. A 3.9 kb *Eco*RI 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.

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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