Comparative and Functional Genomic Studies of

*Histophilus somni (Haemophilus somnis)*

Shivakumara Siddaramappa

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

Doctor of Philosophy

in

Biomedical and Veterinary Sciences

Dr. Thomas J. Inzana, Chair
Dr. Stephen M. Boyle
Dr. Xiang-Jin Meng
Dr. Biswarup Mukhopadhyay
Dr. Brett M. Tyler

Monday, April 9 2007
Blacksburg, Virginia, United States of America

Keywords: *Haemophilus, Histophilus*, Genome,
Bacteriophage, Plasmid, LuxS, Biofilm, Restriction-Modification

Copyright © 2007, Shivakumara Siddaramappa
Comparative and Functional Genomic Studies of

Histophilus somni (Haemophilus somnus)

Shivakumara S. Siddaramappa

ABSTRACT

Histophilus somni is a commensal of the mucosal surfaces of respiratory and reproductive tracts of cattle and sheep. However, as an opportunistic pathogen, H. somni can cause diseases such as pneumonia, myocarditis, abortion, arthritis, and meningo-encephalitis. Previously, several virulence factors/mechanisms had been identified in H. somni of which the phase-variable lipooligosaccharide, induction of host cell apoptosis, intraphagocytic survival, and immunoglobulin Fc binding proteins were well characterized. To further understand the biological properties of H. somni, the genomes of pneumonia strain 2336 and preputial strain 129Pt have been sequenced. Using the genome sequence data and comparative analyses with other members of the Pasteurellaceae, putative genes that encode proteases, restriction-modification enzymes, hemagglutinins, glycosyltransferases, kinases, helicases, and adhesins have been identified in H. somni. Most of the H. somni strain-specific genes were found to be associated with prophage-like sequences, plasmids, and/or transposons. Therefore, it is likely that these mobile genetic elements played a significant role in creating genomic diversity and phenotypic variability among strains of H. somni. Functional characterization of H. somni luxS in the genomic context revealed that the gene encodes S-ribosylhomocysteinase that can complement biosynthesis of AI-2 quorum sensing signal molecules in Escherichia coli DH5α. It was also found that several pathogenic isolates of H. somni form a prominent biofilm and that luxS as well as phosphorylcholine expression can influence biofilm formation by H. somni. In conclusion, comparative analyses of the genomes and functional characterization of putative genes have shed new light on the versatility and evolution of H. somni.
Dedication:

To the Purposeful Ingenuity and Resourceful Mystery of Nature
Acknowledgements

First and foremost, I am grateful to Dr. Thomas J. Inzana for providing the opportunity, space, time, and resources without which the work described in this dissertation would not have been possible. His patience and endurance have been invaluable during my pursuit of the unknown and the abstract.

I am very fortunate in having Dr. Stephen M. Boyle, Dr. Xiang-Jin Meng, Dr. Biswarup Mukhopadhyay, and Dr. Brett M. Tyler as members of my PhD Advisory Committee. Their unconditional support and selfless advice have helped me navigate the labyrinths of my dissertation research. I place on record here my sincere appreciation for them.

I would like to thank Dr. Jean F. Challacombe (DOE Joint Genome Institute) and Dr. David W. Dyer (University of Oklahoma Health Sciences Center) for their overarching role in sequencing the genomes of *Histophilus somni* strains. I also thank Dr. Daphne Y. Rainey (Virginia Bioinformatics Institute at Virginia Tech) for helping me with genome annotation and comparative analyses.

I am indebted to Dr. Roger J. Avery, Dr. Ludeman A. Eng, and Dr. John C. Lee for providing the administrative, financial, and technical support for my academic endeavors. I express my gratitude to Dr. Karen P. DePauw and Dr. Gerhardt G. Schurig for their leadership and vision for graduate education.

I thank Dr. Ann M. Stevens (Virginia Tech) and Dr. Daniel C. Stein (University of Maryland) for providing the *Vibrio harveyi* and *Escherichia coli* reporter strains. I also thank Terry Lawrence and Jerry Baber for their assistance with artwork, photography, and printing.

I cherish the friendship and collegiality of Dr. Michael D. Howard, Gretchen E. Berg, Kristin M. Knight, Rajiv Balyan, Dr. Manas Mandal, Dr. Alison J. Duncan, Cheryl E. Ryder, Dr. Shaadi F. Elswaifi, Anna E. Champion, Dr. Indra Sandal, Xiaoshan Wang, Gerald L. Snider, Parthiban Rajasekharan, Naveen Surendran, and Dr. Abey Bandera.

Financial support from the USDA-CSREES (Initiative for Future Agriculture and Food Systems Grant no. 2001-52100-11314) and the Virginia Agricultural Experiment Station is gratefully acknowledged.
TABLE OF CONTENTS

Abstract ii
Dedication iii
Acknowledgements iv

CHAPTER I

*Histophilus somni* (*Haemophilus somnus*)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>1</td>
</tr>
<tr>
<td>Species characteristics</td>
<td>2</td>
</tr>
<tr>
<td>Classification</td>
<td>5</td>
</tr>
<tr>
<td>Geographic distribution</td>
<td>6</td>
</tr>
<tr>
<td>Clinical diseases</td>
<td>7</td>
</tr>
<tr>
<td>Virulence attributes</td>
<td>9</td>
</tr>
<tr>
<td>Phase variation of lipooligosaccharide</td>
<td>10</td>
</tr>
<tr>
<td>Phosphorylcholination of lipooligosaccharide</td>
<td>12</td>
</tr>
<tr>
<td>Sialylation of lipooligosaccharide</td>
<td>14</td>
</tr>
<tr>
<td>Apoptosis of bovine endothelial cells</td>
<td>15</td>
</tr>
<tr>
<td>Toll-like receptor activation</td>
<td>16</td>
</tr>
<tr>
<td>Resistance to intracellular killing</td>
<td>17</td>
</tr>
<tr>
<td>Induction of bovine platelet aggregation</td>
<td>20</td>
</tr>
<tr>
<td>Immunoglobulin- and transferrin-binding proteins</td>
<td>21</td>
</tr>
<tr>
<td>Host immune response</td>
<td>22</td>
</tr>
<tr>
<td>Vaccination</td>
<td>24</td>
</tr>
<tr>
<td>Conclusions</td>
<td>25</td>
</tr>
<tr>
<td>References</td>
<td>27</td>
</tr>
<tr>
<td>CHAPTER II</td>
<td>A Comparative Perspective on the Genomes of <em>Histophilus somni</em> Strains 2336 and 129Pt</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Abstract</td>
<td>41</td>
</tr>
<tr>
<td>Introduction</td>
<td>42</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>46</td>
</tr>
<tr>
<td>Results</td>
<td>51</td>
</tr>
<tr>
<td>Discussion</td>
<td>98</td>
</tr>
<tr>
<td>References</td>
<td>104</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER III</th>
<th>Characterization of Plasmids from <em>Histophilus somni</em> and Construction of an <em>Escherichia coli–Histophilus somni</em> Shuttle Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>110</td>
</tr>
<tr>
<td>Introduction</td>
<td>111</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>113</td>
</tr>
<tr>
<td>Results</td>
<td>117</td>
</tr>
<tr>
<td>Discussion</td>
<td>138</td>
</tr>
<tr>
<td>References</td>
<td>143</td>
</tr>
<tr>
<td>Appendix 3.01</td>
<td>146</td>
</tr>
<tr>
<td>Appendix 3.02</td>
<td>147</td>
</tr>
</tbody>
</table>
LIST OF MULTIMEDIA OBJECTS

LIST OF FIGURES

CHAPTER I

Figure 1.01: *H. somni* strain 2336 colonies 3
Figure 1.02: *H. somni* strain 2336 colonies 3
Figure 1.03: *H. somni* strain 2336 pigment 3
Figure 1.04: *H. somni* strain 2336 colony 4
Figure 1.05: *H. somni* strain 2336 colonies 4
Figure 1.06: *H. somni* strain 2336 colonies 4
Figure 1.07: Dairy cattle population 6
Figure 1.08: Beef cattle population 6
Figure 1.09: Diseases caused by *H. somni* 8

CHAPTER II

Figure 2.01: *H. somni* strain 2336 chromosome 53
Figure 2.02: *H. somni* strain 129Pt chromosome 54
Figure 2.03: GenePlot comparison 56
Figure 2.04: GenePlot comparison 56
Figure 2.05: GenePlot comparison 56
Figure 2.06: Mummer comparison 57
Figure 2.07: TaxPlot comparison 58
Figure 2.08: TaxPlot comparison 58
Figure 2.09: Fha locus I 82
Figure 2.10: Fha locus II 83
Figure 2.11: Fha locus III 84
Figure 2.12: Fha locus IV 85
Figure 2.13: Comparison of FhaB homologs 86-88
Figure 2.14: Comparison of FhaB homologs 89
Figure 2.15: Comparison of FhaC homologs 90
Figure 2.16: Tbp locus 92
Figure 2.17: *H. somni* strain 2336 subtilisin gene 94
Figure 2.18: Prophage region 96
Figure 2.19: Prophage region 97
Figure 2.20: Prophage region 97

**CHAPTER III**

Figure 3.01: Plasmid profiles of *H. somni* strains 117
Figure 3.02: Circular map of pHS649 120
Figure 3.03: pHS649, p9L, and p57/98 homology 121
Figure 3.04: Comparison of RepA homologs 122
Figure 3.05: pHS649 SSO and DSO regions 123
Figure 3.06: pHS649 stem-loop like structures 124
Figure 3.07: Circular map of pHS649S 125
Figure 3.08: pHS649S in *E. coli* 125
Figure 3.09: Intermediate forms of pHS649S 126
Figure 3.10: Circular map of pHS649SS 127
Figure 3.11: pHS649SS in *E. coli* 127
Figure 3.12: Circular map of pHS129 130
Figure 3.13: pHS129 ori regions

Figure 3.14: Comparison of RepB homologs

Figure 3.15: pHS649SS in *H. somni*

Figure 3.16: Plasmid compatibility

**CHAPTER IV**

Figure 4.01: Principle of AI-2 bioassay

Figure 4.02: Packard luminescence counter

Figure 4.03: *H. somni luxS* map

Figure 4.04: ClustalW alignment of *luxS* ORFs

Figure 4.05: ClustalW alignment of LuxS homologs

Figure 4.06: *H. somni luxS* cloning strategy

Figure 4.07: *H. somni luxS* PCR amplification

Figure 4.08: *V. harveyi* luminescence induction

Figure 4.09: *V. harveyi* luminescence induction

Figure 4.10: Comparison of LuxP homologs

Figure 4.11: Comparison of LuxP homologs

Figure 4.12: Biofilm formation by *H. somni*

Figure 4.13: Biofilm formation by *H. somni*

Figure 4.14: Biofilm formation by *H. somni*

Figure 4.15: Biofilm formation by *H. somni*

Figure 4.16: Biofilm formation by *H. somni*

Figure 4.17: Effect of furanone on *H. somni*

Figure 4.18: Effect of furanone on *H. somni*

Figure 4.19: Effect of furanone on *H. somni*

Figure 4.20: *V. harveyi* luminescence induction
CHAPTER V

Figure 5.01: Map of *H. somni* Type I RM locus 193
Figure 5.02: Map of *H. somni* Type II RM locus 195-196
Figure 5.03: PCR amplification of RM genes 197
Figure 5.04: *E. coli* reporter strain AP1-200-9 198
Figure 5.05: *E. coli* reporter strain AP1-200-9 198
Figure 5.06: *E. coli* reporter strain AP1-200-9 198
Figure 5.07: Comparison of Type II MTases 200
Figure 5.08: Methylation by M.HsoI 201
Figure 5.09: Methylation by M.HsoI 201
Figure 5.10: Comparison of Type II REases 203-204
Figure 5.11: Comparison of Type II REases 203-204
Figure 5.12: R.HinfI digestion 206
Figure 5.13: R.Hin4I digestion 207

LIST OF TABLES

CHAPTER I

Table 1.01: ChoP in pathogenic bacteria 12
Table 1.02: *H. somni* vaccines 24

CHAPTER II

Table 2.01: Characteristics of *H. somni* genomes 55
Table 2.02: *H. somni* strain 129Pt prophages 59
Table 2.03: *H. somni* strain 2336 prophages 60
Table 2.04: *H. somni* 129Pt prophage region I 61
Table 2.05: *H. somni* 129Pt prophage region II 61
Table 2.06: *H. somni* 129Pt prophage region III 62
Table 2.07: *H. somni* 129Pt prophage region IV 63
Table 2.08: *H. somni* 129Pt prophage region V 63
Table 2.09: *H. somni* 129Pt prophage region VI 63
Table 2.10: *H. somni* 129Pt prophage region VII 64-66
Table 2.11: *H. somni* 2336 prophage region I 67-69
Table 2.12: *H. somni* 2336 prophage region II 70-72
Table 2.13: *H. somni* 2336 prophage region III 73-74
Table 2.14: *H. somni* 2336 prophage region IV 75
Table 2.15: *H. somni* 2336 prophage region V 76
Table 2.16: *H. somni* 2336 prophage region VI 77
Table 2.17: *H. somni* 2336 prophage region VII 78-79

CHAPTER III

Table 3.01: *H. somni* strains/isolates 113
Table 3.02: Primers for plasmid sequencing 116
Table 3.03: ORFs of pHS649 and pHS129 136
Table 3.04: Comparison of plasmids 137

CHAPTER IV

Table 4.01: Comparison of *luxS* and LuxS 158
Table 4.02: Comparison of LuxS homologs 162

CHAPTER V

Table 5.01: Primers for RM genes 190
Table 5.02: pSC-A plasmids containing RM genes 197