Histophilus somni Genomics and Genetics

Shivakumara Siddaramappa

Abstract *Histophilus somni* is a commensal and an opportunistic bacterial pathogen associated with multisystemic diseases in cattle and sheep. Some strains of *H. somni* isolated from the genital tract of cattle are biochemically and serologically similar to the pathogenic strains, but are relatively innocuous. Several virulence factors/mechanisms have been identified in *H. somni*, of which the phase-variable lipooligosaccharide, induction of apoptosis of host cells, intraphagocytic survival, and immunoglobulin Fc-binding proteins have been well characterized. The genomes of *H. somni* pneumonia strain 2336 and preputial strain 129Pt have also been sequenced, and comparative analyses of these genomes have provided novel insights into the role of horizontal gene transfer in the evolution of the respective strains. Continued analyses of the genomes of *H. somni* strains and comparing them to the newly sequenced genomes of other bacteria facilitated the identification of a putative integrative and conjugative element (designated ICE*Hso*2336) encoding tetracycline resistance. Comparative genomics also showed that the uptake signal sequence (5′-AAGTGCGGT) of *Haemophilus influenzae* is abundant in *H. somni* and provided a genetic basis for the recalcitrance of some strains of this species to natural transformation. The post-genomic era for *H. somni* offered an opportunity for the functional characterization of genes identified by computational methods. This opportunity has been realized to a great extent by transcriptomic studies that have identified several small noncoding RNAs and new genes. These new discoveries and developments are expected to stimulate further in-depth investigations of *H. somni*, especially from the systems biology viewpoint.

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1 Introduction to Genomic Analyses

The terms "genomics" and "genomic methods" describe "the molecular and bioinformatics techniques that employ all or part of the genome to answer a question about an organism or a group of organisms" (Carruthers et al. 2012). Genomics has immense applications in the quest to understand nature, and comparative microbial genomics is an indispensable tool for molecular pathogenic bacteriology. The first complete genome sequence from a free-living organism was that of *Haemophilus influenzae* strain Rd KW20 (Fleischmann et al. 1995), a close relative of *H. somni*. This pioneering work at the erstwhile Institute for Genomic Research (TIGR) popularized the concept of whole-genome random sequencing by the "shotgun" approach. Since the completion of the first bacterial genome sequence, thousands of bacterial and archaeal genomes have been sequenced and annotated using novel tools and techniques. The genomes of several species of *Pasteurellaceae* have also been sequenced, and whole-genome comparisons have provided new insights into the physiology and evolution of members within this very important bacterial family (Challacombe and Inzana 2008).

Numerous in vitro and in vivo studies during the pre-genomic era have shed light on the differences in virulence properties and genetic traits between *H. somni* pathogenic isolates from sick animals and commensal isolates from the genital tract (Corbeil et al. 1995). *H. somni* pneumonia strain 2336 (NCBI taxonomy ID 228400) and preputial strain 129Pt (NCBI taxonomy ID 205914) have been phenotypically well characterized in the laboratory and utilized in several comparative studies (Corbeil et al. 1997; Inzana et al. 1992, 2002). The genomes of these two strains have been completely sequenced and compared (Challacombe et al. 2007; Siddaramappa et al. 2011). This chapter will provide an overview of the pre-genomic investigations, comparative genomic analyses, and post-genomic studies of *H. somni* strains.

2 Comparative Genomics

Several temperate bacteriophages that infect strains of *H. influenzae* have been purified and described (Williams et al. 2002). However, temperate bacteriophages that infect strains of *H. somni* remain to be isolated and characterized. Nevertheless, prophages and their associated sequences appear to be rife in the genome of *H. somni* strain 2336, but less abundant in the genome of strain 129Pt (Siddaramappa et al. 2011), indicating that the natural repertoire of bacteriophages that potentially infect some strains of *H. somni* strains could be large. Furthermore, a large portion of strain-specific sequences occurring in strains 2336 and 129Pt appear to be due to prophages and their associated sequences (Siddaramappa et al. 2011). Although the Mu-like prophage (FluMu) found in *H. influenzae* strain Rd KW20 is absent in the genomes of *H. somni* strains, the genome of *H. somni* strain 2336 contains a prophage that appears to be partially related to the *Mannheimia haemolytica* serotype A1 lysogenic bacteriophage ϕMhaA1-PHL101.

In addition to the prophages and their associated sequences, the genomes of *H. somni* strains contain several genomic islands that appear to be unrelated to each other (Siddaramappa et al. 2011). A genomic island that is homologous to ICE*Hin1056*, which is a 59,393-bp integrative and conjugative element (~39 % G+C) containing genes encoding ampicillin, chloramphenicol, and tetracycline resistance in *H. influenzae* type b strain 1056, has also been identified in *H. somni* strain 2336 (Mohd-Zain et al. 2004). The genomic island of *H. somni* strain 2336 was more precisely delineated upon comparison with ICE*Pmu1*, which is an integrative and conjugative element (~42 % G+C) containing genes encoding resistance to multiple antibiotics in *Pasteurella multocida* strain 36950 (Michael et al. 2012). This genomic island of *H. somni* strain 2336 appears to be a putative integrative and conjugative element and is referred to as ICE*Hso*2336 (~40.5 % G+C). An integrative and conjugative element (ICE*Mh1*, ~40 % G+C) containing genes encoding resistance to multiple antibiotics and closely related to ICE*Pmu1* is also present in *M. haemolytica* strain 42548 (Eidam et al. 2015). Whereas the nucleotide identity between ICE*Mh1* and ICE*Hin1056* is only ~70 %, the nucleotide identity between ICE*Mh1*, ICE*Pmu1*, and ICE*Hso*2336 is ~99 %.

Furthermore, ICE*Pmu1* and ICE*Mh1* are integrated site-specifically into $tRNA^{Leu}$ in the chromosomes of *P. multocida* strain 36950 and *M. haemolytica* strain 42548, respectively (Eidam et al. 2015; Michael et al. 2012). A comparison of these loci as well as ICE*Hso*2336 indicated that each element contains 11-bp (5′-GATTTTGAATC) terminal direct repeats and an 86-bp $tRNA^{Leu}$ at the right terminus (Fig. 1a). Although ICE*Pmu1* is smaller in size than ICE*Mh1* by ~10,000 bp, it contains more antimicrobial resistance genes than the latter (Eidam et al. 2015; Michael et al. 2012). As reported previously, ICE*Hso*2336 contains the tetracycline repressor gene *tetR* and the tetracycline resistance gene *tetH* (Michael et al. 2012; Mohd-Zain et al. 2004; Siddaramappa et al. 2011), and *H. somni* strain 2336 is resistant to tetracycline (MIC 8 μg/ml) (Ueno et al. 2014). A schematic map of ICE*Hso*2336 is shown in Fig. 1b, and a comparison of the ORFs that occur

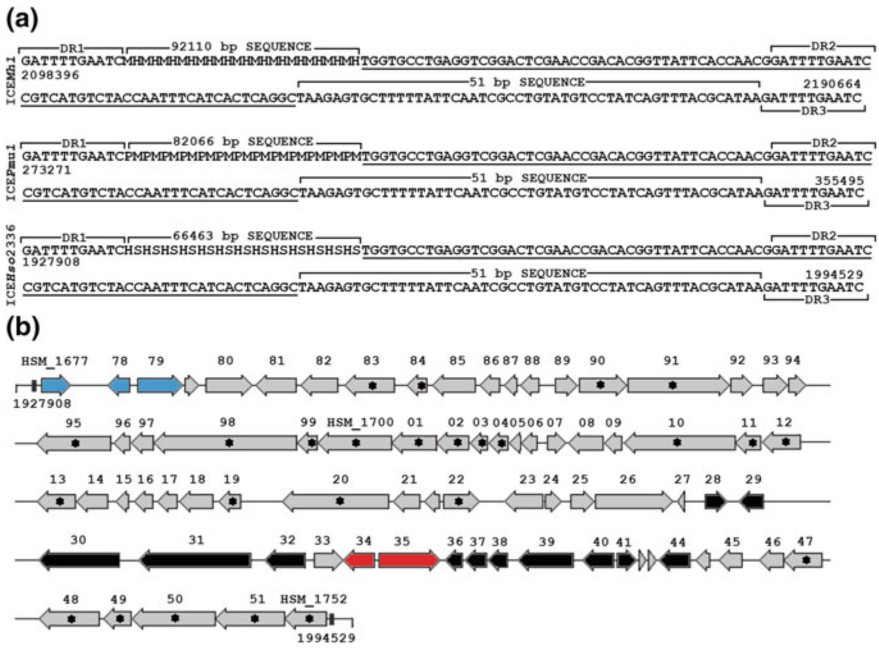

Fig. 1 **a** Comparison of ICE*Mh1*, ICE*Pmu1*, and ICE*Hso*2336. Each ICE contains 11-bp terminal direct repeats (DR1 and DR3) and an additional direct repeat (DR2) within the 86-bp $tRNA^{Leu}$ gene (underlined). A 51-bp sequence occurs between the $tRNA^{Leu}$ gene and DR3. These features are identical among the three ICEs. The sequence between DR1 and the $tRNA^{Leu}$ gene (92110 bp in ICE*Mh1*, 82,066 bp in ICE*Pmu1*, and 66463 bp in ICE*Hso*2336) contains genes that distinguish the three ICEs. Numbers above/below the maps (e.g., 2098396 and 2190664) indicate nucleotide positions within the respective chromosomes. **b** Schematic map of ICE*Hso*2336. Terminal direct repeats (DR1 and DR3) shown in Fig. 1a are indicated by vertical black bars. *Arrows* represent ORFs found within ICE*Hso*2336 and compared in Table 1. *Blue arrows* represent ORFs that have orthologs in ICE*Pmu1*, but not in ICE*Mh1*. *Gray arrows* represent ORFs that have orthologs in ICE*Pmu1* and ICE*Mh1*. *Black arrows* represent ORFs that have no orthologs in ICE*Pmu1* and ICE*Mh1*. *Red arrows* represent *tetR* and *tetH* (have orthologs in ICE*Pmu1* and ICE*Mh1*). Gray arrows containing asterisks represent ORFs that have full-length or partial homologs in *H. somni* strain 129Pt

among ICE*Pmu1*, ICE*Mh1*, and ICE*Hso*2336 is shown in Table 1. Although these elements are closely related, they are not identical and it is evident from Table 1 that they display a mosaic structure with alternating conserved and variable regions. In particular, ICE*Hso*2336 lacked 22 ORFs found in ICE*Pmu1*, and 13 of these 22 ORFs are also absent in ICE*Mh1*. In contrast, ICE*Pmu1* and ICE*Mh1* lack 12 ORFs found in ICE*Hso*2336. Interestingly, *H. somni* strain 129Pt lacks an analogous ICE, but contains short stretches of homologous sequences. Not surprisingly, most of the ORFs identified in ICE*Pmu1* and/or ICE*Hso*2336 have distant homologs outside of the *Pasteurellaceae*.

Table 1 Comparison of the ORFs that occur among ICE*Pmu1*, ICE*Mh1*, and ICE*Hso*2336

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_02680, 192 aa	Hypothetical protein	HSM_1677, 192 aa, 100 %	*Mannheimia varigena*, AHG79487, 192 aa, 99 %	*Streptococcus mutans*, EMC55849, 193 aa, 42 %
Pmu_02690, 145 aa	Putative phage transposase	HSM_1678, 145 aa, 100 %	*Mannheimia varigena*, AHG79486, 94 aa, 100 %	None
Pmu_02700, 299 aa	Tyrosine recombinase-1 family protein (phage integrase)	HSM_1679, 299 aa, 100 %	*Mannheimia varigena*, AHG79485, 303 aa, 100 %	*Salmonella enterica*, ESG49884, 308 aa, 40 %
Pmu_02710, 307 aa	ISApl1 transposase	HSM_1680, 324 aa, 99 %	ICE*Mh1*, AGK02702, 201 aa, 99 %	*Streptococcus pyogenes*, EIK41786, 331 aa, 57 %
Pmu_02740, 271 aa	Aminoglycoside 3'-phosphotransferase protein	None	ICE*Mh1*, AGK02696, 271 aa, 100 %	*Acinetobacter baumannii*, KHW21245, 271 aa, 99 %
Pmu_02760, 278 aa	Aminoglycoside 6'-phosphotransferase protein	None	ICE*Mh1*, AGK02697, 276 aa, 100 %	*Klebsiella pneumoniae*, AHG50664, 279 aa, 100 %
Pmu_02770, 252 aa	Aminoglycoside 3'-phosphotransferase protein	None	ICE*Mh1*, AGK02698, 286 aa, 99 %	*Vibrio cholerae*, EHI02781, 252 aa, 99 %
Pmu_02780, 234 aa	Dihydropteroate synthase	None	*Bibersteinia trehalosi*, AGH37389, 342 aa, 100 %	*Escherichia coli*, EMD02302, 282 aa, 100 %
Pmu_02790, 430 aa	ISCR21 transposase	None	*Bibersteinia trehalosi*, AGH37390, 430 aa, 100 %	*Geobacter* sp. *GSS01*, KIE42276, 430 aa, 97 %
Pmu_02820, 404 aa	Florfenicol/chloramphenicol efflux protein	None	*Bibersteinia trehalosi*, AGH37387, 404 aa, 99 %	*Escherichia coli*, AAS16362, 404 aa, 99 %
Pmu_02830, 101 aa	LysR transcriptional regulator	None	*Actinobacillus pleuropneumoniae*, KIE87619, 101 aa, 100 %	*Vibrio cholerae*, AAV84894, 101 aa, 99 %

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_02840, 497 aa	ISCR2 transposase	None	*Bibersteinia trehalosi*, AGH37386, 357 aa, 99 %	*Shigella flexneri*, NP_838055, 497 aa, 100 %
Pmu_02850, 301 aa	rRNA (adenine-N6-)-methyltransferase	None	*Bibersteinia trehalosi*, AHG85539, 234 aa, 100 %	*Morganella morganii*, CDK68643, 303 aa, 99 %
Pmu_02880, 254 aa	Tyrosine recombinase-2 family protein (phage integrase)	None	ICE*Mh1*, AGK02704, 254 aa, 100 %	*Salmonella enterica*, YP_002149427, 308 aa, 46 %
Pmu_02890, 659 aa	Integrating conjugative element relaxase	None	ICE*Mh1*, AGK02705, 659 aa, 100 %	*Pseudomonas xanthomarina*, CEG51019, 625 aa, 37 %
Pmu_02900, 265 aa	Putative type I restriction–modification system methyltransferase subunit	HSM_1681, 265 aa, 99 %	ICE*Mh1*, AGK02706, 265 aa, 100 %	*Providencia stuartii*, AIN62209, 269 aa, 41 %
Pmu_02910, 248 aa	Hypothetical protein	HSM_1682, 248 aa, 100 %	ICE*Mh1*, AGK02707, 248 aa, 100 %	*Neisseria gonorrhoeae*, KDM99755, 259 aa, 39 %
Pmu_02920, 324 aa	Hypothetical protein	HSM_1683[a], 324 aa, 100 %	ICE*Mh1*, AGK02708, 324 aa, 100 %	*Yersinia ruckeri*, KGX82889, 332 aa, 46 %
Pmu_02930, 135 aa	Hypothetical protein	HSM_1684[a], 135 aa, 100 %	ICE*Mh1*, AGK02709, 135 aa, 100 %	None
Pmu_02940, 280 aa	Hypothetical protein	HSM_1685, 280 aa, 100 %	ICE*Mh1*, AGK02710, 280 aa, 100 %	*Alcanivorax* sp. 19-m-6, KGD61388, 280 aa, 35 %
Pmu_02950, 131 aa	Hypothetical protein	HSM_1686, 131 aa, 100 %	ICE*Mh1*, AGK02711, 131 aa, 100 %	None

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_02960, 81 aa	Hypothetical protein	HSM_1687, 81 aa, 100 %	ICE*Mh1*, AGK02712, 81 aa, 100 %	None
Pmu_02970, 117 aa	Hypothetical protein	HSM_1688, 117 aa, 100 %	ICE*Mh1*, AGK02713, 117 aa, 100 %	None
Pmu_02980, 147 aa	Integrating conjugative element protein	HSM_1689, 147 aa, 100 %	ICE*Mh1*, AGK02714, 147 aa, 100 %	*Serratia* sp. ATCC 39006, ESN64104, 134 aa, 38 %
Pmu_02990, 313 aa	Integrating conjugative element protein	HSM_1690[a], 313 aa, 100 %	ICE*Mh1*, AGK02715, 313 aa, 100 %	*Klebsiella oxytoca*, KFC43925, 310 aa, 55 %
Pmu_03000, 670 aa	Integrating conjugative element protein	HSM_1691[a], 670 aa, 100 %	ICE*Mh1*, AGK02716, 670 aa, 100 %	*Salmonella enterica*, ESG40339, 461 aa, 44 %
Pmu_03010, 142 aa	Hypothetical protein	HSM_1692, 142 aa, 100 %	ICE*Mh1*, AGK02717, 142 aa, 100 %	None
Pmu_03020, 156 aa	Hypothetical protein	HSM_1693, 156 aa, 100 %	ICE*Mh1*, AGK02718, 156 aa, 100 %	*Leptotrichia* sp. ERK50264, 146 aa, 36 %
Pmu_03030, 113 aa	Hypothetical protein	HSM_1694, 113 aa, 100 %	ICE*Mh1*, AGK02719, 113 aa, 100 %	None
Pmu_03040, 490 aa	TraG-like domain-containing protein	HSM_1695[a], 490 aa, 100 %	ICE*Mh1*, AGK02720, 490 aa, 100 %	*Salmonella enterica*, ESG40341, 498 aa, 46 %
Pmu_03050, 105 aa	Hypothetical protein	HSM_1696, 105 aa, 100 %	ICE*Mh1*, AGK02721, 105 aa, 100 %	None
Pmu_03060, 142 aa	Hypothetical protein	HSM_1697, 142 aa, 100 %	ICE*Mh1*, AGK02722, 142 aa, 100 %	None
Pmu_03070, 945 aa	Conjugative transfer ATPase TraC-like protein	HSM_1698[a], 945 aa, 100 %	ICE*Mh1*, AGK02723, 945 aa, 100 %	*Salmonella enterica*, ESG40327, 902 aa, 56 %

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_03080, 132 aa	Conjugative transfer region lipoprotein	HSM_1699[a], 132 aa, 100 %	ICE*Mh1*, AGK02724, 132 aa, 100 %	*Pseudomonas brassicacearum,* AHL34884, 138 aa, 53 %
Pmu_03090, 484 aa	Integrating conjugative element protein	HSM_1700[a], 484 aa, 100 %	ICE*Mh1*, AGK02725, 484 aa, 100 %	*Salmonella enterica*, ESG40325, 468 aa, 47 %
Pmu_03100, 294 aa	Integrating conjugative element protein	HSM_1701[a], 294 aa, 100 %	ICE*Mh1*, AGK02726, 294 aa, 100 %	*Xenorhabdus bovienii*, CDH06738, 277 aa, 53 %
Pmu_03110, 214 aa	Integrating conjugative element protein	HSM_1702[a], 214 aa, 100 %	ICE*Mh1*, AGK02727, 214 aa, 100 %	*Klebsiella pneumoniae*, ESL45356, 208 aa, 48 %
Pmu_03120, 121 aa	Conjugative transfer region protein	HSM_1703[a], 121 aa, 100 %	ICE*Mh1*, AGK02728, 121 aa, 100 %	*Salmonella enterica*, ABX22958, 117 aa, 43 %
Pmu_03130, 129 aa	Integrating conjugative element protein	HSM_1704[a], 129 aa, 100 %	ICE*Mh1*, AGK02729, 129 aa, 100 %	None
Pmu_03140, 77 aa	Hypothetical protein	HSM_1705, 78 aa, 100 %	ICE*Mh1*, AGK02730, 77 aa, 100 %	*Salmonella enterica*, EHL38285, 78 aa, 36 %
Pmu_03150, 108 aa	Integrating conjugative element protein	HSM_1706[a], 108 aa, 100 %	ICE*Mh1*, AGK02731, 108 aa, 100 %	*Salmonella enterica*, ESH30146, 98 aa, 45 %
Pmu_03160, 127 aa	Hypothetical protein	HSM_1707, 127 aa, 100 %	ICE*Mh1*, AGK02732, 127 aa, 100 %	None
Pmu_03170, 231 aa	Integrating conjugative element membrane protein	HSM_1708, 231 aa, 100 %	ICE*Mh1*, AGK02733, 231 aa, 100 %	*Halomonas* sp., ERL52507, 234 aa, 45 %
Pmu_03180, 117 aa	Hypothetical protein	HSM_1709, 117 aa, 100 %	ICE*Mh1*, AGK02734, 117 aa, 100 %	None

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_03190, 733 aa	Conjugative coupling factor TraD	HSM_1710[a], 733 aa, 99 %	ICE*Mh1*, AGK02735, 733 aa, 100 %	*Salmonella enterica*, ESG40317, 703 aa, 61 %
Pmu_03200, 168 aa	Integrating conjugative element protein	HSM_1711[a], 168 aa, 100 %	ICE*Mh1*, AGK02736, 168 aa, 100 %	*Pseudomonas xanthomarina*, CEG54628, 180 aa, 42 %
Pmu_03210, 256 aa	Putative lysozyme-like protein	HSM_1712[a], 256 aa, 100 %	ICE*Mh1*, AGK02737, 256 aa, 100 %	*Acinetobacter calcoaceticus*, EOQ63901, 723 aa, 48 %
Pmu_03220, 257 aa	Integrating conjugative element protein	HSM_1713[a], 257 aa, 100 %	ICE*Mh1*, AGK02738, 257 aa, 100 %	*Escherichia coli*, EFM51825, 235 aa, 44 %
Pmu_03230, 209 aa	Integrating conjugative element protein	HSM_1714, 209 aa, 100 %	ICE*Mh1*, AGK02739, 209 aa, 100 %	*Pseudomonas* sp. 12M76, CEA04059, 183 aa, 37 %
Pmu_03240, 86 aa	Hypothetical protein	HSM_1715, 86 aa, 100 %	ICE*Mh1*, AGK02740, 86 aa, 100 %	*Phycisphaera mikurensis*, BAM05377, 90 aa, 49 %
Pmu_03250, 120 aa	Hypothetical protein	HSM_1716, 120 aa, 100 %	ICE*Mh1*, AGK02741, 120 aa, 100 %	*Neisseria gonorrhoeae*, YP_003600430, 113 aa, 32 %
Pmu_03260, 135 aa	Hypothetical protein	HSM_1717, 135 aa, 99 %	ICE*Mh1*, AGK02742, 135 aa, 100 %	*Acinetobacter* sp. NIPH 899, ENV00920, 119 aa, 42 %
Pmu_03270, 226 aa	Hypothetical protein	HSM_1718, 226 aa, 99 %	ICE*Mh1*, AGK02743, 226 aa, 100 %	None
Pmu_03280, 143 aa	Hypothetical protein	HSM_1719[a], 143 aa, 100 %	ICE*Mh1*, AGK02744, 143 aa, 100 %	*Pseudomonas savastanoi*, EFW82260, 184 aa, 59 %

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_03290, 683 aa	DNA topoisomerase III	HSM_1720[a], 683 aa, 100 %	ICE*Mh1*, AGK02745, 683 aa, 100 %	*Bordetella hinzii*, KCB25912, 671 aa, 51 %
Pmu_03300, 172 aa	Hypothetical protein	HSM_1721, 172 aa, 100 %	ICE*Mh1*, AGK02746, 172 aa, 100 %	None
Pmu_03310, 94 aa	Hypothetical protein	None	ICE*Mh1*, AGK02747, 94 aa, 100 %	None
Pmu_03320, 236 aa	Hypothetical protein	HSM_1722[a], 236 aa, 100 %	ICE*Mh1*, AGK02748, 236 aa, 100 %	*Proteus mirabilis*, KGA90870, 241 aa, 40 %
Pmu_03330, 255 aa	Aldo/keto reductase	HSM_1723, 255 aa, 100 %	ICE*Mh1*, AGK02749, 255 aa, 100 %	*Flavobacterium subsaxonicum*, KGO90979, 381 aa, 55 %
Pmu_03340, 106 aa	Hypothetical protein	HSM_1724, 106 aa, 100 %	ICE*Mh1*, AGK02750, 106 aa, 100 %	None
Pmu_03350, 151 aa	Hypothetical protein	HSM_1725, 51 aa, 100 %	ICE*Mh1*, AGK02751, 151 aa, 100 %	*Pseudomonas aeruginosa*, ERV17255, 161 aa, 55 %
Pmu_03360, 515 aa	Multicopper oxidase protein	HSM_1726, 515 aa, 100 %	ICE*Mh1*, AGK02752, 515 aa, 100 %	*Kingella denitrificans*, EGC16775, 99 %
None	Putative transcriptional regulator, MerR family	HSM_1728, 134 aa	*Mannheimia varigena*, AHG73107, 149 aa, 100 %	*Kingella kingae*, EGK11561, 129 aa, 62 %
None	Protein of unknown function	HSM_1729, 105 aa	*Mannheimia varigena*, AHG79433, 145 aa, 100 %	*Neisseria wadsworthii*, EGZ49932, 129 aa, 61 %
None	Multicopper oxidase type 3	HSM_1730, 534 aa	*Mannheimia varigena*, AHG73105, 534 aa, 99 %	*Kingella denitrificans*, EGC16775, 515 aa, 60 %

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
None	Heavy metal translocating P-type ATPase	HSM_1731, 730 aa	*Mannheimia varigena*, AHG73102, 717 aa, 99 %	*Bordetella bronchiseptica*, KAK67234, 831 aa, 86 %
None	Protein of unknown function	HSM_1732, 248 aa	*Mannheimia varigena*, AHG73100, 248 aa, 100 %	*Neisseria wadsworthii*, EGZ46421, 270 aa, 94 %
Pmu_03390, 172 aa	Hypothetical protein	HSM_1733, 172 aa, 100 %	ICE*Mh1*, AGK02755, 172 aa, 100 %	*Acinetobacter baumannii*, EXS20166, 314 aa, 100 %
Pmu_03400, 207 aa	Tetracycline repressor protein (TetR)	HSM_1734, 207 aa, 100 %	ICE*Mh1*, AGK02756, 207 aa, 100 %	*Proteus hauseri*, EST57920, 207 aa, 96 %
Pmu_03410, 209 aa	Aminoglycoside 2”-O-adenyltransferase protein	None	None	*Escherichia coli*, YP_009082244, 195 aa, 99 %
Pmu_03420, 263 aa	Aminoglycoside 3”-O-adenyltransferase protein	None	*Pasteurella aerogenes*, YP_006961169, 258 aa, 91 %	*Salmonella enterica*, ABL95942, 263 aa, 98 %
Pmu_03440, 275 aa	Beta-lactamase OXA-2 protein	None	None	*Salmonella enterica*, NP_511223, 275 aa, 100 %
Pmu_03450, 234 aa	IS26 transposase	None	*Bibersteinia trehalosi*, AGH37398, 238 aa, 99 %	*Escherichia coli*, YP_006953879, 239 aa, 100 %
Pmu_03460, 491 aa	Macrolide efflux protein	None	*Bibersteinia trehalosi*, AGH37396, 491 aa, 100 %	*Citrobacter freundii*, NP_775053, 491 aa, 100 %
Pmu_03470, 294 aa	Macrolide 2’-phosphotransferase protein	None	*Bibersteinia trehalosi*, AGH37397, 294 aa, 99 %	*Acinetobacter baumannii*, YP_001736317, 294 aa, 99 %

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Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
Pmu_03480, 234 aa	IS26 transposase	None	*Bibersteinia trehalosi*, AGH37398, 238 aa, 100 %	*Salmonella enterica*, YP_209349, 240 aa, 100 %
Pmu_03490, 207 aa	Tetracycline repressor protein (TetR)	HSM_1734, 207 aa, 100 %	ICE*Mh1*, AGK02756, 207 aa, 100 %	*Proteus hauseri*, EST57920, 207 aa, 96 %
Pmu_03500, 400 aa	Tetracycline efflux protein, class H (TetH)	HSM_1735, 400 aa, 99 %	ICE*Mh1*, AGK02757, 400 aa, 99 %	*Gilliamella apicola*, KES17253, 400 aa, 99 %
Pmu_03510, 436 aa	Transposase	None	ICE*Mh1*, AGK02758, 436 aa, 100 %	*Escherichia coli*, ELJ62457, 436 aa, 92 %
Pmu_03520, 100 aa	Hypothetical protein	None	ICE*Mh1*, AGK02760, 100 aa, 100 %	None
Pmu_03530, 62 aa	Hypothetical protein	None	ICE*Mh1*, AGK02761, 62 aa, 100 %	*Vibrio fluvialis*, EPP21553, 71 aa, 73 %
None	Small multidrug resistance protein	HSM_1736, 110 aa	*Mannheimia varigena*, AHG73095, 110 aa, 100 %	*Cardiobacterium valvarum*, EHM53491, 99 aa, 63 %
None	Transcriptional regulator, MarR	HSM_1737, 149 aa	*Mannheimia varigena*, AHG73094, 149 aa, 100 %	*Clostridium] clostridioforme*, ENZ04504, 152 aa, 39 %
None	Protein of unknown function	HSM_1738, 123 aa	*Mannheimia varigena*, AHG73093, 123 aa, 100 %	*Neisseria flavescens*, EER56534, 123 aa, 64 %
None	Conserved hypothetical protein	HSM_1739, 366 aa	*Mannheimia varigena*, AHG73092, 366 aa, 100 %	*Tannerella forsythia*, AEW20145, 367 aa, 49 %
None	Cation efflux protein	HSM_1740, 204 aa	*Mannheimia varigena*, AHG73091, 172 aa, 100 %	*Lautropia mirabilis*, EFV94656, 212 aa, 55 %

(continued)

Table 1 (continued)

ICE*Pmu1* locus tag, protein	Annotation	*H. somni* strain 2336 (ICE*Hso*2336), ortholog locus tag, protein, identity	Closest homolog within *Pasteurellaceae*; (other than *H. somni*), species/ICE, locus tag, protein, identity	Closest homolog outside *Pasteurellaceae*, species, locus tag, protein, identity
None	Putative transcriptional regulator, MerR	HSM_1741, 132 aa	*Mannheimia varigena*, AHG73090, 132 aa, 100 %	*Enhydrobacter aerosaccus*, EEV23030, 150 aa, 43 %
None	Alcohol dehydrogenase zinc-binding domain protein	HSM_1744, 202 aa	*Mannheimia varigena*, AHG73087, 201 aa, 100 %	*Pseudomonas* sp., KEY89048, 330 aa, 64 %
Pmu_03540, 150 aa	Single-stranded DNA-binding protein	HSM_1745, 150 aa, 100 %	ICE*Mh1*, AGK02762, 150 aa, 100 %	*Cycloclasticus* sp., EPD13323, 154, 60 %
Pmu_03550, 161 aa	Hypothetical protein	HSM_1746, 161 aa, 100 %	ICE*Mh1*, AGK02763, 161 aa, 100 %	*Salmonella enterica*, CCF89832, 164 aa, 31 %
Pmu_03560, 252 aa	Integrating conjugative element protein	HSM_1747[a], 252 aa, 100 %	ICE*Mh1*, AGK02764, 252 aa, 100 %	*Citrobacter youngae*, EFE07328, 232 aa, 40 %
Pmu_03570, 396 aa	Hypothetical protein	HSM_1748[a], 396 aa, 99 %	ICE*Mh1*, AGK02765, 396 aa, 100 %	*Salmonella enterica*, ABX22929, 377 aa, 37 %
Pmu_03580, 185 aa	Hypothetical protein	HSM_1749[a], 185 aa, 100 %	ICE*Mh1*, AGK02766, 185 aa, 100 %	*Salmonella enterica*, ESJ18378, 179 aa, 38 %
Pmu_03590, 1548 aa	Integrating conjugative element ParB family protein	HSM_1750[a], 548 aa, 100 %	ICE*Mh1*, AGK02767, 548 aa, 100 %	None
Pmu_03600, 428 aa	Replicative DNA helicase	HSM_1751[a], 453 aa, 99 %	ICE*Mh1*, AGK02768, 453 aa, 99 %	*Klebsiella pneumoniae*, EMI36970, 432 aa, 49 %
Pmu_03610, 274 aa	Chromosome partitioning ATPase	HSM_1752[a], 274 aa, 100 %	ICE*Mh1*, AGK02769, 274 aa, 100 %	*Salmonella enterica*, ESJ18374, 294 aa, 46 %

[a]Full-length or partial homolog is present in *H. somni* strain 129Pt

It is interesting to note that *M. haemolytica* strain 42548, *P. multocida* strain 36950, and *H. somni* strain 2336 were isolated from cases of naturally occurring bovine respiratory tract infections in different parts of the USA (Pennsylvania, Nebraska, and Washington, respectively) in different years (2007, 2005, and 1980s, respectively), but harbor closely related genomic elements containing antibiotic resistance determinants. Multidrug-resistant isolates of these respiratory pathogens appear to be more common among animals in bovine feedlots (Klima et al. 2014). Furthermore, horizontal transfer of ICEs that mediate antibiotic resistance from *M. haemolytica* and *H. somni* to *P. multocida*, and from *P. multocida* to *Escherichia coli*, has been demonstrated (Klima et al. 2014). It is possible that these ICEs have a common evolutionary origin, and indiscriminate use of antibiotics favors their preservation and dispersal in the field.

3 Comparative Transcriptomics

Computational gene prediction at best provides a "first pass" structural annotation of genomes and has many limitations, which could be overcome using experimental approaches that involve the analyses of the transcriptome. Attempts have been made to obtain a high-resolution transcriptome map of *H. somni* strain 2336 using the Illumina RNA-Seq technology (Kumar et al. 2012). Comparison of the transcriptome map of strain 2336 with the computationally annotated genome facilitated the identification of 94 small noncoding RNA (sRNA) of various sizes (70–695 bp, average G+C content 39.3 %). A vast majority of these sRNA (82 of 94) were reported to be novel (unidentified in previous bacterial transcriptome studies) and proposed to play roles in housekeeping and virulence, in addition to gene regulation. Sequence analyses of the 94 sRNA indicated that 31 were specific to strain 2336, 41 were specific to strains 2336 and 129Pt, 11 had homologs only in the genomes of *P. multocida*, *H. influenzae*, and *H. parainfluenzae*, and 11 had homologs in the genomes of other distantly related bacteria (Kumar et al. 2012). Furthermore, the start sites of five predicted genes (HSM_0031, 0525, 0789, 1019, and 1729) were corrected using the RNA-Seq data and comparison with other phylogenetically related homologs.

Genome annotation had predicted that putative proteins encoded by HSM_0603, 0748, 1385, 1666, and 1744 (hypothetical protein, α-L-fucosidase, 3-hydroxydecanoyl-ACP dehydratase, DNA damage-inducible protein, and alcohol dehydrogenase, respectively) were shorter than their homologs in other species. RNA-Seq data of strain 2336 showed the presence of full-length mRNA for these genes and confirmed that the putative proteins were truncated at the *N*-terminus due to either frameshift mutations (for HSM_1385 and 1744) or non-functional start codons (for the other three genes) (Kumar et al. 2012). Analyses of the RNA-Seq data indicated that 1636 of the 1980 predicted protein-coding genes were transcribed and there were 278 operons consisting of 730 genes in *H. somni* strain 2336 (Kumar et al. 2012).

4 Plasmids and Shuttle Vectors

Plasmid-borne resistance to multiple antibiotics is a relatively common feature among some members of the *Pasteurellaceae*. Isolates of *H. somni* resistant to tetracycline and harboring *tetH*, albeit lacking plasmids, have been cultured from nasal swabs of feedlot calves from Alberta, Canada (D'Amours et al. 2011). Furthermore, plasmid profiling as a means of identification and characterization of field isolates of *H. somni* has been reported (Appuhamy et al. 1998; Fussing and Wegener 1993). Efforts have also been made toward deciphering and describing the complete nucleotide sequences of plasmids from *H. somni* strains (Izadpanah et al. 2001; Siddaramappa et al. 2006). All four *H. somni* circular plasmids whose sequences have been deciphered/described are referred to as cryptic plasmids since they lack the genes that encode functions other than those necessary for their own replication (Izadpanah et al. 2001; Siddaramappa et al. 2006). Interestingly, the largest *H. somni* circular plasmid that has been completely sequenced (pHS129, 5178 bp) appears to be a dimer (Siddaramappa et al. 2006), and the natural occurrence of such plasmid dimers among bacteria is relatively rare.

The possibility of using native or non-native plasmids, after suitable modifications, as shuttle vectors that function in *E. coli* and *H. somni* has been explored. *H. somni* strain HS91 was transformed with plasmid pD70Kan^R, which is derived from *M. haemolytica* plasmid pD70 (Briggs and Tatum 2005). Interestingly, in vitro modification of pD70Kan^R using a commercially available *HhaI* methylase significantly improves the transformation efficiency (Briggs and Tatum 2005). Furthermore, *H. somni* strain 129Pt, which contains plasmid pHS129, can be transformed with pLS88, which is a broad-host-range plasmid purified from *Haemophilus ducreyi* (Sanders et al. 1997). In vivo modification of pLS88 using the recombination-deficient *H. influenzae* strain DB117 improves the transformation efficiency (Sanders et al. 1997).

H. somni strain 129Pt has also been transformed with a modified version of *H. somni* circular plasmid pHS649 (Siddaramappa et al. 2006). Derivatives of pLS88 that transform *H. somni* with a higher efficiency (e.g., pNS3K) have also been developed using kanamycin resistance as the selectable marker (Sandal et al. 2008). Therefore, it appears that pHS129 and pLS88 do not belong to the same incompatibility group, as are pHS129 and pHS649. The possibility of improving these vectors or other forms of pLS88 (such as pLSSK and pLSKS) (Wood et al. 1999) for efficient transformation of *H. somni* strains remains to be explored.

5 Mutagenesis

Although chemical mutagenesis is a popular technique in bacterial genetics and ethyl methanesulfonate has been used to obtain non-capsulated mutants of *Actinobacillus pleuropneumoniae* (Inzana et al. 1993), it has not been widely used

in other members of the *Pasteurellaceae*. Transformation and mutagenesis of strains of *H. somni* using exogenous DNA molecules is difficult, at least in part due to an omnipresent restriction–modification system.

Molecular genetic analyses of *H. somni* were invigorated following the demonstration that in vitro or in vivo modification improves the transformation efficiency of shuttle plasmids for *H. somni* (Briggs and Tatum 2005; Sanders et al. 1997). Successful transformation of *H. somni* strain 129Pt with a putative virulence-associated gene of *H. somni* strain 2336, and the stable expression of the gene in the transformed strain, demonstrated the utility of *H. somni* preputial isolates for genetic analyses (Sanders et al. 1997). Furthermore, transformation of strain 129Pt was also used to demonstrate that *lob1* is involved in lipooligosaccharide (LOS) biosynthesis in *H. somni* and that the 5′-$(CAAT)_n$ repeats within *lob1* are involved in LOS phase variation (McQuiston et al. 2000) (see chapter on "The Many Facets of Lipooligosaccharide as a Virulence Factor for *Histophilus somni*"). These studies used a commercial electroporator to introduce heterologous DNA into *H. somni* rendered electrocompetent by growth in brain–heart infusion broth or Columbia broth and washing the bacterial pellets with 272 mM sucrose solution (Briggs and Tatum 2005; McQuiston et al. 2000; Sanders et al. 1997).

A non-replicative suicide plasmid methylated in vitro by *HhaI* methylase was used for mutagenesis of a *H. somni* strain 738 DNA locus involved in LOS biosynthesis by homologous recombination-mediated allelic exchange (Wu et al. 2000). The mutant strains had an altered LOS profile in comparison with the wild-type strain, indicating that *lob2A* could be involved in LOS biosynthesis (Wu et al. 2000). However, the prototype mutant strain (*H. somni* 738-*lob2A1::Km*) could not be complemented using shuttle vector pLSlob2A, reportedly due to inefficient electrotransformation (Wu et al. 2000) (see chapter on "The Many Facets of Lipooligosaccharide as a Virulence Factor for *Histophilus somni*").

A combination of methylation in vivo using the *H. influenzae* cloning strain DB117 and in vitro using *HhaI* methylase has been shown to improve the transformation efficiency of plasmids for *H. somni* strain 8025 (Sanders et al. 2003). A fivefold increase in transformation efficiency is observed after plasmids derived from *H. somni* strain 8025 are reintroduced into the same strain by electroporation (Sanders et al. 2003), indicating that the restriction–modification systems among *H. influenzae* and *H. somni* strains could be different. Furthermore, homologous recombination-mediated allelic exchange was used for partial deletion of a locus encoding high molecular weight immunoglobulin-binding proteins (HMW IgBPs) in *H. somni* strain 8025 (Sanders et al. 2003). A significant difference ($p < 0.001$) in the adherence of the mutant or wild-type strain to bovine pulmonary artery endothelial cells was also reported (Sanders et al. 2003). Of interest is that both *lob1* and the gene encoding for HMW IgBPs contain the *H. influenzae* uptake signal sequence (see Sect. 6).

A temperature-sensitive plasmid was developed to obtain in-frame, unmarked *aroA* deletion mutants of *H. somni* (Tatum and Briggs 2005). *M. haemolytica* native plasmid pD70 was modified by inserting the Tn903 kanamycin resistance cassette and the modified plasmid ($pD70Kan^R$) mutagenized using hydroxylamine. A single

base-pair mutation from G to A at position 301 within the origin of replication renders this plasmid temperature sensitive. The *aroA* gene from *H. somni* was amplified by PCR and cloned into the temperature-sensitive plasmid pGA301*ori*C to create pTs*Hsaro*C. An in-frame deletion was engineered within pTs*Hsaro*C to create the replacement plasmid pTs*Hs*Δ*aroA*C (Tatum and Briggs 2005). This replacement plasmid is methylated in vitro using *HhaI* methylase, electroporated into *H. somni* strain 2336, and recovered at the permissive temperature of 30 °C for 2 h on medium containing 50 μg/ml kanamycin. The plates are then incubated at the non-permissive temperature (41 °C) for 16 h to select for single-crossover mutants containing the temperature-sensitive replacement plasmid integrated into the chromosome by homologous recombination (Tatum and Briggs 2005). Single-crossover mutants are cultured in broth without kanamycin at the permissive temperature for 16 h to facilitate a second crossover event and plasmid excision. This process is repeated twice, and bacteria from the third-pass culture are streaked onto plates without kanamycin. The plates are incubated at 37 °C for 16 h, and colonies are further replica-plated with or without kanamycin selection. After incubation at 37 °C, kanamycin-sensitive colonies are selected and the absence of the kanamycin gene is tested by PCR. Deletion of *aroA* is also confirmed by PCR (Tatum and Briggs 2005).

A non-replicative suicide plasmid methylated in vitro using *HhaI* methylase can also be used for complete deletion of the *ibpA* open reading frame encoding HMW IgBPs in *H. somni* strain 2336 by homologous recombination-mediated allelic exchange (Hoshinoo et al. 2009). The isogenic mutant strain was less cytotoxic than wild-type strain 2336 for bovine FBM-17 macrophage-like cells, murine J774.1 macrophage-like cells, and bovine primary monocyte cells (Hoshinoo et al. 2009). Although wild-type strain 2336 significantly compromised the ability of murine J774.1 macrophage-like cells and bovine primary monocyte cells to phagocytize microspheres, the isogenic mutant strain had no such effect, indicating that IbpA (specifically the Fic region; see chapter on "*Histophilus somni* Surface Proteins") of *H. somni* may play a role in pathogenesis (Hoshinoo et al. 2009).

Homologous recombination-mediated exchange of genes encoding the major outer membrane protein (MOMP) between *H. somni* strains 129Pt and 2336 has been described (Ueno et al. 2014). Since plasmid-based cloning of the *H. somni* gene encoding MOMP proved difficult, a vector-free strategy that utilizes the direct electroporation of PCR-amplified, *HhaI*-methylated linear DNA into *H. somni* was developed (Ueno et al. 2014). Following allelic exchange, strain 129Pt stably expresses the gene encoding MOMP from strain 2336 (HSM_1447, *ompH*/OmpH, 1443 bp/380 aa) and strain 2336 stably expresses the gene encoding MOMP from strain 129Pt (HS_0971, *ompH*/OmpH, 951 bp/316 aa), and the proteins can be detected by Western and dot blots using strain-specific anti-MOMP monoclonal antibodies. Furthermore, strains 129Pt and 2336 stably express a chimeric gene encoding MOMP (due to combining parts of genes encoding MOMPs from the two strains) after allelic exchange, and the fusion proteins can be detected using strain-specific anti-MOMP monoclonal antibodies in Western and dot blots (Ueno et al. 2014). The serum susceptibilities of strain 129Pt expressing HSM_1447 and

strain 129Pt expressing the fusion protein (containing portions of HSM_1447 at the *C*-terminus) are significantly greater than those of the wild type (Ueno et al. 2014). This is not surprising since the genomes of strains 129Pt and 2336 differ from each other, and the genes encoding the OmpH homologs have only 56 % identity.

To overcome the inherent low efficiency of transformation and recombination of non-replicative suicide plasmids used for allelic exchange in *H. somni*, improved methods of mutagenesis need to be developed. Mutagenesis of *H. somni* using a commercially available transposon (Sandal et al. 2009) represents a significant step in this direction. Electroporation of *H. somni* strain 2336 yields up to 100 kanamycin-resistant colonies per 20 ng of the EZ-Tn5™ <KAN-2> Tnp Transposome™ (Epicentre, Madison, WI). Of 500 transposon mutants of *H. somni* strain 2336 screened for biofilm formation using the crystal violet assay, 55 formed either more or less biofilm than the wild-type strain. Of the several transposon mutants confirmed to produce less biofilm than the wild-type strain by scanning electron microscopy, six contained a transposon insertion in a region of the *ibpA* gene that encodes a putative filamentous hemagglutinin. This indicates that filamentous hemagglutinins, which are important attachment factors in other pathogenic bacteria [such as *Bordetella* (Villarino Romero et al. 2014)], likely contribute to *H. somni* biofilm formation and possibly pathogenesis (Sandal et al. 2009). Mutagenesis of *H. somni* strain 2336 genes putatively encoding S-ribosylhomocysteinase (*luxS*), universal stress protein E (*uspE*), major facilitator transport protein, and a protein of unknown function has also been achieved using the EZ-Tn5™ <KAN-2> Tnp Transposome™ (Sandal et al. 2009; Shah et al. 2014). Interestingly, both *luxS* and *uspE* mutants are attenuated in an acute septicemia mouse model, whereas only the *uspE* mutant is deficient in biofilm formation (Shah et al. 2014).

6 Natural Transformation

The ability of bacteria to internalize chromosomal fragments and/or plasmids under natural conditions is referred to as *competence*. Competence is proposed to be regulated by biochemical as well as environmental cues, and the purposes for internalizing DNA within the host cell could be non-genetic (e.g., nutrition) or genetic (e.g., transformation) (Mell and Redfield 2014). Although most naturally competent bacteria are indiscriminate in DNA internalization, members of the *Pasteurellaceae* and the *Neisseriaceae* are known to prefer conspecific DNA. The preferential internalization of conspecific DNA by members of these two families appears to be facilitated by short uptake signal sequences (Mell and Redfield 2014). In *H. influenzae*, the uptake signal sequence is a nonamer (5′-AAGTGCGGT, or its reverse complement), and comparative genomic analyses have demonstrated the abundance of this sequence in *Actinobacillus actinomycetemcomitans*, *P. multocida*, and *H. somni* (Bakkali et al. 2004; Redfield et al. 2006).

Although several members of the *Pasteurellaceae* are believed to be competent, only *H. influenzae*, *A. actinomycetemcomitans*, and *A. pleuropneumoniae* have been shown to undergo natural transformation under laboratory conditions (Redfield et al. 2006). In other species, the lack of competence or transformation in the laboratory is believed to be due to the failure to mimic native conditions and/or dysfunctional genetic systems (Redfield et al. 2006). Notably, *H. somni* strains 2336 and 129Pt lack a *comD* homolog and appear to encode a shortened ComE homolog. Since it has been demonstrated that functionality of each gene within the *com* operon is essential for transformation of *H. influenzae* (Carruthers et al. 2012), it could be presumed that *H. somni* strains 2336 and 129Pt are non-transformable. This appears to be valid in the case of strain 129Pt since it fails to be transformed when plasmid pNS3K (Sandal et al. 2008), genomic DNA from a *lob2A* mutant of strain 738 (Wu et al. 2000), or genomic DNA from transposon mutants of *luxS* or *uspE* of strain 2336 is used (Shah et al. 2014). However, strain 2336 can be transformed with a low efficiency when plasmid pNS3K or genomic DNA from a *lob2A* mutant of strain 738 is used. Nevertheless, strain 2336 fails to transform when genomic DNA from transposon mutants of *luxS* or *uspE* is used. Moreover, *H. somni* strains 649, 8025, and M14-622 also fail to transform when genomic DNA from a *lob2A* mutant of strain 738 is used (Shah et al. 2014). Therefore, it is likely that *H. somni* strains differ in their competency/transformability, and this could be due to the lack of specific *com* genes and/or the variety of restriction–modification systems that occur in this species (Briggs and Tatum 2005; Siddaramappa et al. 2011). Differences in competence and transformation have also been observed among strains of *H. influenzae* lacking specific *com* genes (Maughan and Redfield 2009). Furthermore, transformation of *H. influenzae* with restriction endonuclease-digested conspecific DNA is dependent on fragment size (Beattie et al. 1982), and it has been hypothesized that restriction endonucleases released by lysed cells may cut donor DNA fragments destined for uptake and reduce recombination efficiency (Mell and Redfield 2014).

7 Conclusions

Biochemical and genetic studies in the pre-genomic era firmly establish *H. somni* as a potent opportunistic pathogen. Complete genome sequencing reveals the pathogenic repertoire of this species, and comparative genomic analyses facilitate the identification of chromosomal regions that resemble the pathogenicity islands of other virulent bacteria. One such pathogenicity island has now been identified as ICE*Hso*2336 and appears to represent a classical horizontally transferred element. Transcriptome analyses indicate that ~80 % of the predicted genes of *H. somni* strain 2336 are readily transcribed, and ~44 % of these genes are operonic. Furthermore, electrotransformation of *H. somni* appears to be more efficient than natural transformation. In addition, genetic manipulation of *H. somni* is achievable through either suicide plasmid-based homologous recombination (targeted mutagenesis) or transposomes

(random mutagenesis), and several plasmids are now available that can serve as shuttle vectors. Future investigations of *H. somni* are expected to be guided by the principles, technologies, and developments discussed in this chapter.

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