

# Complete Genome Sequence of *Haemophilus somnus* (*Histophilus somni*) Strain 129Pt and Comparison to *Haemophilus ducreyi* 35000HP and *Haemophilus influenzae* Rd<sup>†</sup>

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***Haemophilus somnus* can be either a commensal of bovine mucosal surfaces or an opportunistic pathogen. Pathogenic strains of *H. somnus* are a significant cause of systemic disease in cattle. We report the genome sequence of *H. somnus* 129Pt, a nonpathogenic commensal preputial isolate, and the results of a genome-wide comparative analysis of *H. somnus* 129Pt, *Haemophilus influenzae* Rd, and *Haemophilus ducreyi* 35000HP. We found unique genes in *H. somnus* 129Pt involved in lipooligosaccharide biosynthesis, carbohydrate uptake and metabolism, cation transport, amino acid metabolism, ubiquinone and menaquinone biosynthesis, cell surface adhesion, biosynthesis of cofactors, energy metabolism, and electron transport. There were also many genes in common among the three organisms. Our comparative analyses of *H. somnus* 129Pt, *H. influenzae* Rd, and *H. ducreyi* 35000HP revealed similarities and differences in the numbers and compositions of genes involved in metabolism, host colonization, and persistence. These results lay a foundation for research on the host specificities and niche preferences of these organisms. Future comparisons between *H. somnus* 129Pt and virulent strains will aid in the development of protective strategies and vaccines to protect cattle against *H. somnus* disease.**

*Haemophilus somnus* (*Histophilus somni*), a member of the family Pasteurellaceae (47), can be either a commensal of bovine mucosal surfaces or an opportunistic pathogen. Commensal *H. somnus* strains colonize and reside relatively harmlessly in the upper respiratory and reproductive tracts, while pathogenic strains spread systemically and cause diseases such as pneumonia, thrombotic meningoencephalitis, myocarditis, septicemia, arthritis, and abortion (10, 34, 41, 42). Here, we report the genome sequence of *H. somnus* 129Pt, a commensal preputial isolate. We compared *H. somnus* 129Pt with the finished genomes of *Haemophilus influenzae* Rd, an avirulent laboratory strain that can invade certain human epithelial cell lines in culture (12), and *Haemophilus ducreyi* 35000HP, which causes the human sexually transmitted disease chancroid. *H. somnus* 129Pt and *H. influenzae* Rd are moderately related (14, 25), do not cause disease, and colonize mucosal niches in different hosts. Phylogenetic analyses have shown that *H. ducreyi* is not closely related to members of the genus *Haemophilus* (1, 14, 30) but is a member of the Pasteurellaceae that colonizes genital mucosal surfaces. Its surface lipooligosaccha-

rides (LOS) are similar in structure to those from *Haemophilus* and *Neisseria* strains (8), and it occupies a (mucosal) niche similar to that of *H. somnus* but in a different host. Therefore, the identification of common genes that these organisms share as well as genes unique to each organism may provide some clues about their host specificities and niche preferences.

Through a genome-wide comparative analysis of *H. somnus* 129Pt, *H. influenzae* Rd, and *H. ducreyi* 35000HP, we found similarities and differences among these organisms in terms of the numbers and compositions of genes involved in metabolism, host colonization, and persistence. Our results provide a foundation of information about the commensal 129Pt strain of *H. somnus*, revealing clues about its preference for the bovine urogenital tract environment. Our work will be useful in future genomic comparisons with pathogenic isolates to gain insight into virulence mechanisms and potentially lead to new strategies for the prevention and control of diseases caused by *H. somnus*.

## MATERIALS AND METHODS

**Sequencing of the *H. somnus* 129Pt genome.** The random shotgun method was used for sequencing of the genome of *H. somnus* 129Pt. Large-insert (40-kb), medium-insert (8-kb), and small-insert (3-kb) random sequencing libraries were sequenced for this genome project, with an average success rate of 96% and average high-quality read lengths of 685 nucleotides. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible misassemblies were corrected with Dupfinisher (C. Han, unpublished data) or a transposon bomb of bridging clones (EZ-Tn5 <P6Kyori/KAN-2> Tnp

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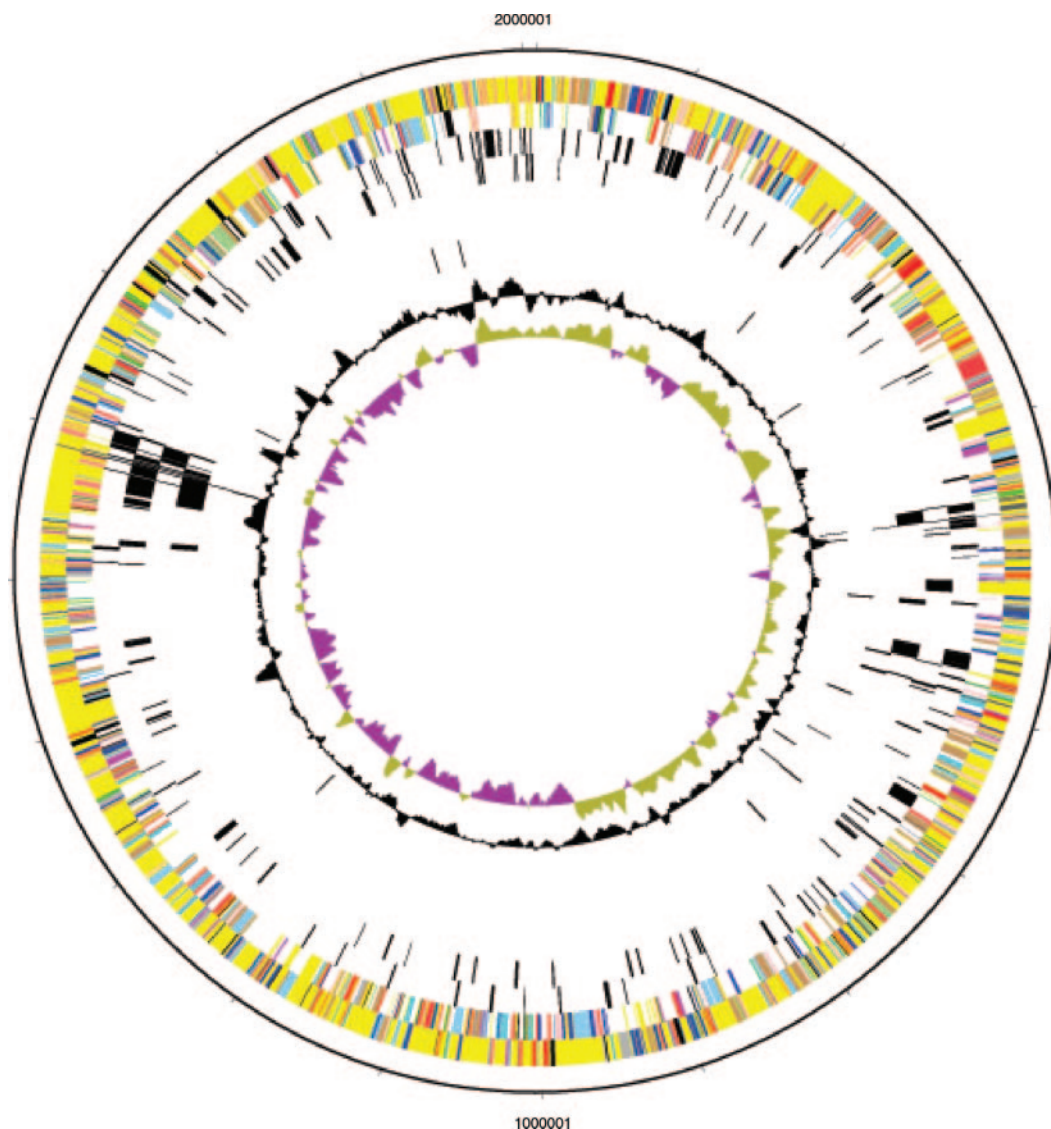


FIG. 1. Circular representation of the *H. somnus* 129Pt genome. The outermost two circles indicate start sites of genes and assigned functional categories. Circle 1 consists of forward-strand gene products. Circle 2 consists of reverse-strand gene products. Colors represent the following functional categories: amino acid biosynthesis (magenta); biosynthesis of cofactors, prosthetic groups, and carriers (red); cell envelope (dark salmon); cellular processes (light gray); central intermediary metabolism (orange); conserved hypothetical and hypothetical (yellow); DNA metabolism (light blue); energy metabolism (cyan); fatty acid and phospholipid metabolism (pale salmon); *Haemophilus*-specific protein (salmon); other categories (dark gray); protein synthesis and protein fate (blue); purines, pyrimidines, nucleosides, and nucleotides (green); regulatory functions (black); transcription and translation (light green); transport and binding proteins (brown); and unassigned (palest salmon). Circles 3 and 4, unique genes; circles 5 and 6, prophage genes; circles 7 and 8, insertion elements; circle 9, G+C content; circle 10, GC bias ( $G - C/G + C$ ). Khaki indicates values  $>1$ , and purple indicates values  $<1$ .

transposome kit; Epicenter Biotechnologies). Gaps between contigs were closed by editing, custom primer walks, or PCR amplification. The completed genome sequence of *H. somnus* 129Pt contains 59,147 reads, achieving an average of 18-fold sequence coverage per base with an error rate of less than 1 in 100,000.

**Annotation.** Gene predictions were obtained using Glimmer (13, 60), and tRNAs were identified using tRNAscan-SE (46). Basic analysis of the gene predictions was performed by comparing coding sequences against the Pfam, BLOCKS, COGS, and ProDom databases. Gene definitions and functional classes were added manually by a team of annotators by using BLAST results in addition to information from the basic analysis.

**Sequence analysis.** To obtain lists of common and unique genes in the *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd genomes, we used blastp with a cutoff of  $1e-5$  to obtain best hits for the proteins of each genome

blasted against each other. We used the blast2gi program from the Seals package (69) to format the BLAST results in tabular form.

**Nucleotide sequence accession numbers.** The sequences of the *H. somnus* 129Pt chromosome and plasmid pHS129 have been deposited in the GenBank database under accession numbers CP000436 and CP000019.

## RESULTS AND DISCUSSION

**General genome features.** The 2.0-Mb *H. somnus* 129Pt chromosome (Fig. 1) contained 1,844 predicted coding sequences (CDSs), 5 rRNA operons, and 50 tRNAs. The average

TABLE 1. General features of the *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd genomes

Parameter	Value for organism		
	<i>H. somnus</i> 129Pt	<i>H. ducreyi</i> 35000HP	<i>H. influenzae</i> Rd <sup>a</sup>
Size (Mb)	2.0	1.7	1.8
Plasmid	pHS129 (5.2 kb)	None	None
Coding sequence (Mb)	1.8	1.5	1.6
Coding sequence (%)	89.5	86.6	89
GC%	37	38	38
Average ORF size (bp)	980	828	915
Total no. of CDSs	1,844	1,781	1,743
No. of CDSs with assigned function	1,313	1,092	1,007
No. of conserved hypothetical CDSs	440	495	347
No. of hypothetical CDSs	61	130	389
No. of tRNAs	50	45	54
No. of rRNA operons	5	6	6
No. of possible pseudogenes	15 <sup>b</sup>	19 <sup>b</sup>	62 <sup>c</sup>

<sup>a</sup> Data for *H. influenzae* Rd were obtained from reference 21.

<sup>b</sup> Number of genes annotated as fragments and N-terminal, C-terminal, and central regions.

<sup>c</sup> Data were obtained from reference 65 and indicate the number of genes containing frameshifts and early stop codons.

open reading frame (ORF) length was 980 bp, and the overall coding density was 89.5%. The overall GC content was 37% (Table 1). *H. somnus* 129Pt had five candidate prophage regions (see Table S1 in the supplemental material): HS\_0423 to HS\_0433 spanned 9,814 bases, with a GC content of 31%; HS\_0519 to HS\_0539 spanned 11,509 bases, with a GC content of 35%; HS\_0554 to HS\_0558 spanned 3,289 bases, with a GC content of 33%; HS\_1331 to HS\_1337 spanned 4,076 bases, with a GC content of 36%; and HS\_1365 to HS\_1425 spanned 52,616 bases, with a GC content of 40%. *H. somnus* 129Pt had approximately 15 pseudogenes (see Table S2 in the supplemental material); this number may not include all genes with frameshifts or early stop codons. In *H. somnus* 129Pt, the origin of replication (*oriC*) was located at approximately base 1926721 (within HS\_1702 *atpB* at positions 1927397 to 1926603); the replication terminus region (*terC*) was located at approximately base 953326 (in IGR0751, positions 952932 to 953616 between HS\_0872 and HS\_0873). The *dnaA* gene (HS\_0136) was translocated relative to *oriC* in *H. somnus* 129Pt. IS1016 elements are novel insertion elements present in *H. influenzae* (43). We found five complete and three partial copies of IS1016 in *H. somnus* 129Pt and one in *H. ducreyi* 35000HP (see Table S3 in the supplemental material).

Comparisons of the *H. somnus* 129Pt, *H. influenzae* Rd, and *H. ducreyi* 35000HP chromosomes using ACT (<http://www.sanger.ac.uk/Software/ACT/>) revealed no large regions of synteny. Pairwise BLAST comparisons of the protein sequences from *H. somnus* 129Pt, *H. influenzae* Rd, and *H. ducreyi* 35000HP revealed 319 CDSs unique to *H. somnus* 129Pt, 411 CDSs unique to *H. ducreyi* 35000HP, and 228 CDSs unique to *H. influenzae* Rd (Fig. 2). *H. somnus* 129Pt and *H. ducreyi* 35000HP had 1,091 sequences in common, *H. somnus* 129Pt and *H. influenzae* Rd had 1,242 sequences in common, and *H. ducreyi* 35000HP and *H. influenzae* Rd had 1,113 sequences in common. The three organisms had approximately 983 to 999 genes in common. Genes unique to *H. somnus* 129Pt (see Table S4 in the supplemental material) included genes involved in LOS biosynthesis, carbohydrate uptake and metabolism, cation transport, amino acid metabolism, ubiquinone and menaquinone biosynthesis, cell surface adhesion, biosynthesis of cofactors, energy metabolism, and electron transport. A genome-wide comparative analysis of *H. somnus* 129Pt, *H. influenzae* Rd, and *H. ducreyi* 35000HP revealed similarities and differences in the numbers and compositions of genes involved in various aspects of metabolism and host colonization and persistence.

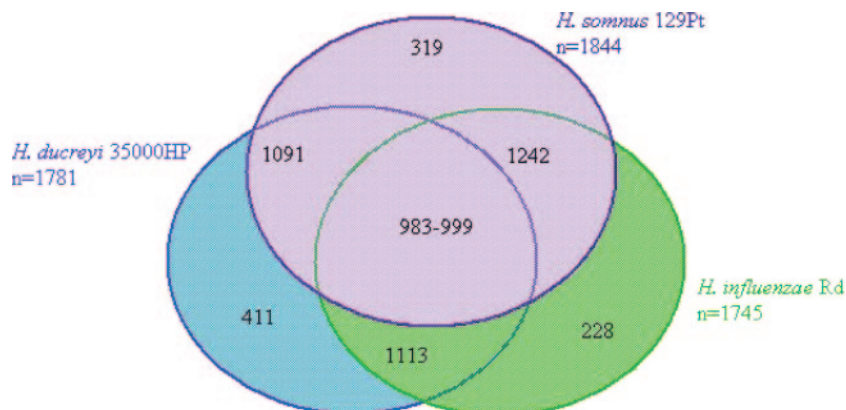


FIG. 2. Common and unique genes in *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd.



**Carbohydrate and amino acid utilization.** *H. somnus* 129Pt had approximately 94 genes involved in carbohydrate utilization, encoding metabolic enzymes or transporters, while *H. ducreyi* 35000HP had approximately 27 genes and *H. influenzae* Rd had about 58 genes (see Table S5 in the supplemental material). The approximate numbers of genes involved in amino acid utilization in *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd were 81, 59, and 103, respectively (see Table S6 in the supplemental material). Our results show that *H. somnus* 129Pt had more genes for carbohydrate utilization than did *H. ducreyi* 35000HP and *H. influenzae* Rd, while both *H. somnus* 129Pt and *H. influenzae* Rd had more genes for amino acid utilization than did *H. ducreyi*. These results suggest that the bovine urogenital tract, in which *H. somnus* 129Pt lives, and the human respiratory tract, where *H. influenzae* lives, contain more abundant nutrient sources than human genitalia, where *H. ducreyi* resides.

**Nucleotide biosynthesis.** *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd all had genes encoding components of the purine biosynthesis pathway. However, the *H. ducreyi* 35000HP genes *purC*, *purD*, *purF*, *purH*, *purK*, and *purL* were fragmented; these genes were each represented by two or three separate adjacent ORFs (see Table S7 in the supplemental material), indicating that they may not be functional. In terms of pyrimidine biosynthesis genes, *H. somnus* 129Pt and *H. ducreyi* 35000HP were missing *carA*, *carB*, *pyrB*, *pyrC*, and *pyrI*, as is *H. influenzae* Rd (65). In *H. influenzae* Rd, added citrulline can salvage both arginine and pyrimidine biosynthesis via the *argI* gene product (65). *H. ducreyi* also had *argI*, so it should be able to use citrulline. Since *H. somnus* 129Pt did not have *argI*, the lack of *carA*, *carB*, *pyrB*, *pyrC*, and *pyrI* explains the need for uracil in the growth medium (35).

**TCA cycle, Embden-Meyerhof, pentose phosphate, and Entner-Doudoroff pathways.** Like *H. influenzae* (65), both *H. somnus* 129Pt and *H. ducreyi* 35000HP lacked genes encoding aerobic respiratory chain enzymes and were missing key enzymes that are part of the oxidative branch of the tricarboxylic acid (TCA) cycle. However, in terms of the missing enzymes, *H. ducreyi* 35000HP and *H. influenzae* Rd were more similar to each other than to *H. somnus* 129Pt, as they were missing the genes encoding citrate synthase, aconitase, and isocitrate dehydrogenase, while *H. somnus* 129Pt was missing only the gene encoding isocitrate dehydrogenase. In terms of the rest of the TCA cycle, all three organisms were missing succinate dehydrogenase as well as the entire glyoxylate bypass. *H. ducreyi* 35000HP was also missing the gene encoding succinyl coenzyme A synthetase, which both *H. somnus* 129Pt and *H. influenzae* Rd had. *H. ducreyi* 35000HP was also missing succinyl coenzyme A synthetase (*sucCD*), while the other two organisms had these genes (HS\_0956 to HS\_0957 and HI1196 to HI1197). *H. somnus* 129Pt, *H. influenzae* Rd, and *H. ducreyi* 35000HP all had the genes *aspC* (HS\_0838, HD1419, and HI1617) and *aspA* (HS\_0466, HD0564, and HI0534), whose products convert oxaloacetate to aspartate and alpha-ketoglutarate (*aspC*) and aspartate to fumarate (*aspA*), thus bypassing the steps catalyzed by the missing GltA, AcnB, and Icd.

These results indicate that *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd (65) have an incomplete, reductive TCA cycle. Many other bacteria have an incomplete TCA cycle, and very few have a complete one (33). This in-

complete reductive type of cycle probably functions primarily in carbon assimilation and the generation of precursor metabolites for biosynthesis; phylogenetic evidence indicates that the original state of the TCA cycle was a reductive biosynthetic pathway (59).

Since *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd were all lacking the complete energy-producing branch of the TCA cycle, they must have other means for energy generation. Both *H. somnus* 129Pt and *H. influenzae* Rd had complete Embden-Meyerhof (glycolysis) pathways. *H. somnus* 129Pt had a homolog of the *Escherichia coli* glucokinase (*glk*) (HS\_0379). *H. influenzae* Rd did not have a homolog of the *E. coli* glucokinase but did have two genes (HI0144 and HI0182) encoding proteins that are weakly homologous to two glucokinases from *Streptomyces coelicolor* A3 (20 to 30% amino acid identity) (65). Based on BLAST sequence comparisons to *E. coli*, *S. coelicolor*, *H. somnus* 129Pt, and *H. influenzae* Rd genes, *H. ducreyi* 35000HP did not appear to have glucokinase at all (see Table S8 in the supplemental material). However, it did have the rest of the glycolysis pathway, so it should be able to convert glucose-6-phosphate to pyruvate. All three organisms had the complete pentose phosphate pathway, but only *H. somnus* 129Pt and *H. influenzae* Rd had the Entner-Doudoroff pathway. In terms of fermentation, all three organisms had the genes necessary for the conversion of pyruvate to lactate, formate, and acetate but not to ethanol (see Table S8 in the supplemental material).

**Ubiquinone and menaquinone biosynthesis.** Chorismate is the starting point for ubiquinone and menaquinone synthesis (49). *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd all had the shikimate pathway for chorismate biosynthesis. In addition, *H. somnus* 129Pt had all of the genes encoding components of both the ubiquinone (*ubiABCDEFGHX*) and menaquinone (*menABCDEFGF*) synthesis pathways, and *H. ducreyi* 35000HP had genes encoding all of the components of the ubiquinone synthesis pathway except chorismate lyase (*ubiC*), which catalyzes the first step in the pathway, the conversion of chorismate to *p*-hydroxybenzoate (see Table S8 in the supplemental material). Without this enzyme, *H. ducreyi* 35000HP cannot synthesize ubiquinone; we were unable to find genes comprising a complete pathway to bypass this step, such as the pathway utilizing chorismate mutase, prephenate dehydrogenase, and phenylalanine ammonia lyase commonly found in plants and studied in *Escherichia coli* mutants (5). *H. influenzae* Rd had none of the genes for ubiquinone biosynthesis (see Table S8 in the supplemental material). Like *H. somnus* 129Pt, both *H. ducreyi* 35000HP and *H. influenzae* Rd had the complete menaquinone biosynthesis pathway.

**NAD biosynthesis.** In culture, *H. somnus* (3) and *H. ducreyi* (48, 53) do not require NAD (V factor) for growth, but *H. influenzae* Rd does (73). This indicates that *H. somnus* may be able to use nicotinamide as a precursor in NAD biosynthesis, as *H. ducreyi* does (48). The *H. ducreyi* 35000HP genome contained two full copies and a fragment of the gene *nadV* (48, 53), which encodes nicotinamide phosphoribosyl transferase (HD1445 fragment, HD1447, and HD1455), which allows strains that possess it to be grown in the presence of nicotinamide (48). *H. somnus* 129Pt had a *nadV* homolog (HS\_0002) with 54% amino acid identity to HD1447 and HD1455. This explains the requirement for nicotinamide in the defined me-

dium for growth of *H. somnus* (35). In *H. ducreyi* 35000HP, the *nadV* gene is part of the pNAD1 plasmid sequence, which is integrated into the chromosome of *H. ducreyi* 35000HP as two tandem copies (HD1440 to HD1455) (53). *H. somnus* 129Pt had one chromosomal copy of *nadV* (HS\_0002) and did not contain integrated copies of the pNAD1 plasmid, as it had none of the other genes encoded by pNAD1.

*H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd did not have the *nadA*, *nadB*, *nadC*, *nadD*, and *nadE* genes, which encode the de novo pathway for the synthesis of NAD from L-aspartate (see Table S9 in the supplemental material). In eukaryotes, the de novo pathway starts with tryptophan, and some bacteria may utilize that pathway rather than the aspartate pathway (44). However, none of these organisms had the eukaryotic tryptophan pathway. The salvage pathway in *H. influenzae* involves a bifunctional protein encoded by the *nadR* gene. *H. somnus* 129Pt had a homolog (HS\_0087) of the *H. influenzae nadR* (HI0763) gene. In *H. ducreyi* 35000HP, *nadR* was split into two ORFS (HD0274 and HD0275), which may not be functional.

**Host colonization and persistence.** Although *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd do not cause systemic infection (51, 62), they do colonize and persist in their hosts. We investigated whether these three organisms shared any genes involved in host colonization and persistence and whether they had any genes in common with invasive strains of *H. influenzae*. Colonization of mucosal surfaces involves the circumvention of mucociliary function so that the clearance of the bacteria is impeded and adherence of bacterial cells to host epithelium (reviewed in references 58 and 72). Interference with mucosal ciliary activity is accomplished by LOS (38) (see Table S10 in the supplemental material).

Successful colonization of mucosal surfaces by bacteria depends on their adherence to host mucosal epithelial cells (58, 72). Evidence indicates that adherence of *Haemophilus* spp. to host cells involves multiple mechanisms including pili and a wide variety of adhesins (reviewed in reference 36). The *H. influenzae* pilus gene cluster (*hifA*, *hifY3*, *hifY2*, *hifY1*, *hifB*, *hifC*, *hifD*, and *hifE*) is present in pathogenic isolates but not in strains like Rd (21, 68). We also did not find similar genes in *H. somnus* 129Pt (see Table S11 in the supplemental material). *H. ducreyi* 35000HP had genes with low similarity (~30% protein sequence identity) to *hifA* and *hifB* (HD0284), *hifC* (HD0283), and *hifD* (HD0281). The protein translations of these genes were also ~30% identical to the type 1 pilus components encoded by *fimA* (HD0281), *fimC* (HD0284), and *fimD* (HD0283) of *E. coli* (39). If not involved in the formation of type 1 pili, these *H. ducreyi* 35000HP genes might be part of a secretion system. The *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd genomes did contain candidate *pilA*, *pilB*, *pilC*, and *pilD* genes (see Tables S11 and S13 in the supplemental material), which encode the components necessary to process and export the PilA subunit of type IV pili (15). However, the *H. somnus* 129Pt *pil* genes were not clustered as in *H. ducreyi* 35000HP and *H. influenzae* Rd.

The *H. somnus* 129Pt and *H. ducreyi* 35000HP *pil* genes were similar in sequence to the *H. influenzae* genes encoding the components of the DNA uptake machinery (see Table S13 in the supplemental material). Therefore, based on sequence similarity alone, it is impossible to know which function is

provided by these *pil* genes in *H. somnus* 129Pt. Evidence indicates that the type IV pilin-like proteins encoded by the *H. influenzae* Rd *pilABCD* genes are necessary for transformation (15). However, it has recently been demonstrated that *H. influenzae* strain 86-028NP expresses type IV pilus-like structures and twitching motility when grown under certain conditions (4). Twitching motility and the presence of type IV pili have not yet been investigated in *H. somnus* 129Pt. In the absence of demonstrated twitching motility, it is likely that the *H. somnus* 129Pt genes function in DNA uptake, as *H. somnus* 129Pt does have genes involved in DNA uptake and transformation. If twitching motility by *H. somnus* is demonstrated in the future, then these components may function in both motility and DNA uptake, as has been described previously for *Neisseria gonorrhoeae* (24).

*H. ducreyi* expresses fine, tangled pili, and the major pilin protein is encoded by the gene *ftpA* (7) (HD0068), which is not present in *H. influenzae* Rd or *H. somnus* 129Pt. In addition, *H. ducreyi* has the *tad* genes, which are responsible for tight adherence to surfaces. The *tad* loci are part of a 15-gene cluster in *H. ducreyi* that includes the *rcpABC* and *ftp* genes, which are necessary for *H. ducreyi* microcolony formation in vitro (55) and may be important for colonization in a variety of environmental niches (40).

As expected, *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd lacked the adhesin genes found in pathogenic strains of *H. influenzae* (see Table S11 in the supplemental material). However, they did have other adhesin genes, which may be involved in colonization and persistence. Most notably, *H. somnus* 129Pt had 12 genes encoding large adhesins, which had low sequence similarity to two filamentous hemagglutinins from *H. ducreyi* 35000HP (HD1156 and HD1505) and three genes from *H. influenzae* Rd (HI1718, HI1685, and HI0383) (see Table S11 in the supplemental material). The protein sequences of these adhesins had C-terminal DsrA-like and Hia-like domains, indicating that they are probably autotransporters, which would use the Sec secretion system to move across the inner membrane (reviewed in reference 11). All three organisms had the *sec* secretion system genes.

Spinola's group previously identified several genes required for *H. ducreyi* infection and colonization of the skin. These genes include *dsrA* (autotransporter adhesin) (6), *lspA1* and *lspA2* (filamentous hemagglutinins) (37), *dltA* (lectin A), *hgbA* (hemoglobin receptor) (2), peptidoglycan-associated lipoprotein (P6) (22), *ncaA* (collagen-binding outer membrane protein) (23), and *tadA* (tight adherence protein A) (63). *H. somnus* 129Pt had genes with significant sequence similarity to *dsrA* (HS\_1543) and P6 (HS\_0263) (see Table S11 in the supplemental material) and also had two ORFS (the result of a frameshift) representing the N-terminal and C-terminal regions of *hgbA* (HS\_0720 and HS\_0721) (see Table S12 in the supplemental material). *H. influenzae* Rd had *hgbA* (HI0661) and P6 (HI0381) genes. *H. somnus* 2336 has a trimeric autotransporter adhesin (11), which had 47% amino acid identity to two genes (HS\_1085 and HS\_1234) in strain 129Pt. In addition, Both *H. influenzae* Rd and *H. ducreyi* 35000HP had *oapA* (HI0330/HD0651) and *oapB* (HI0331/HD0652) genes. *H. somnus* 129Pt had only *oapA* (HS\_1287). OapA is required for *H. influenzae* colonization of the rat nasopharynx (70). OapB may be a lipoprotein (70), but its function is currently unknown.

Therefore, it is likely that many of these genes in *H. somnus* 129Pt and *H. influenzae* Rd play a role in colonization.

The persistence of bacteria at the original site of colonization depends on the ability to evade the immune system and the ability to acquire iron from the host (58, 72). Immune evasion is accomplished in part by phase variation in the structures of surface LOS. The LOS biosynthesis genes in *H. ducreyi*, *H. influenzae*, and *H. somnus* have been studied in detail (28, 50, 62). Table S10 in the supplemental material shows all LOS biosynthetic genes identified in *H. somnus* 129Pt and *H. ducreyi* 35000HP, compared to *H. influenzae* Rd (21).

*H. somnus* 129Pt genes that were not found in *H. influenzae* or *H. ducreyi* included HS\_0116 and HS\_0275, encoding glycosyltransferases. *H. somnus* also has the *lob* locus, consisting of *lob1*, *lob2A*, *lob2B*, *lob2C*, and *lob2D*; the products of these *lob* genes are glycosyltransferases that may be homologs of the *Neisseria lgt* genes responsible for building the lacto-*N*-neotetraose chain (62). As previously reported, strain 129Pt has *lob1* (HS\_0638) and *lob2D* (HS\_0636) but has only the 5' ends of *lob2A* (HS\_0637) and *lob2C* (HS\_0636a). The *lob2B* gene was not present at all. *H. ducreyi* 35000HP had genes annotated as *lgtA* and *lgtB* (similar to *lob2A/lob2B* and *lob2C*, respectively) as well as potential homologs of *lob1* and *lob2D*. *H. influenzae* Rd had *lic2A* (*lob2A* homolog) and genes with similarity to *lob2C* and *lob2D* (see Table S10 in the supplemental material).

The persistence of bacteria in the host may also be facilitated by antigenic variation, such as that which occurs in the outer membrane protein P2 of *H. influenzae* Rd (HI0139), which is immunogenic (58). *H. ducreyi* 35000HP had two copies of the gene encoding P2 (HD1433 and HD1435), but *H. somnus* 129Pt did not have any. *H. ducreyi* 35000HP and *H. influenzae* Rd also had genes encoding P4, which *H. somnus* 129Pt did not have. All three organisms had the genes encoding P1, P5, and P6 (see Table S11 in the supplemental material); this finding is consistent with the observed reactivity of *H. somnus* with a monoclonal antibody (kindly provided by Tim Murphy) to P6 (unpublished data).

In addition to evading the immune system, bacteria must be able to synthesize heme or acquire it and iron from the host in order to survive and persist (reviewed in references 58 and 72). All of these organisms contained genes that should enable them to satisfy their iron requirement (see Table S12 in the supplemental material). Both *H. ducreyi* 35000HP and *H. influenzae* Rd lack the complete pathway for heme biosynthesis (20, 71), while *H. somnus* 129Pt had genes encoding all components of the heme biosynthesis pathway from L-glutamate to protoheme (see Table S12 in the supplemental material). Although *H. influenzae* Rd does not encode the genes required to make heme from 5-amino-levulinate, it does have ferrochetalase (*hemH*), which adds an iron molecule to protoporphyrin IX to make protoheme (71).

We found that *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd had approximately 15, 9, and 17 iron and heme transporter genes, respectively (see Table S12 in the supplemental material). *H. influenzae* can use heme, heme-hemopexin, and iron-bound transferrin as sources of iron as well as iron salts and chelates if protoporphyrin IX is available (71). *H. influenzae* does not produce siderophores (52, 54), can use human transferrin as a sole source of iron (31), and has two genes encoding the transferrin-binding proteins Tbp1 and

Tbp2 (HI0994 [*tbpA*] and HI0995 [*tbpB*], respectively) (26), which facilitate the uptake of iron in a TonB-dependent process (58). *H. ducreyi* does not produce siderophores and cannot use heme-hemopexin (64). *H. ducreyi* can fulfill its iron requirement by using free heme, hemoglobin, catalase, and hemoglobin complexed with haptoglobin but not human transferrin or lactoferrin (45). Since it cannot use transferrin as a source of iron, it is not surprising that *H. ducreyi* 35000HP lacked transferrin-binding protein genes (*tbpA* and *tbpB*). *H. ducreyi* 35000HP also did not have homologs of the *H. influenzae hxuABC* genes (HI0262 to HI0264) for heme-hemopexin utilization. *H. somnus* 129Pt does not make siderophores (56) and did not have homologs of the *hxuABC* genes, indicating that it cannot use heme-hemopexin as a heme source either. *H. somnus* 129Pt had *tbpA* (HS\_0449) and *tbpB* (HS\_0448) genes, encoding the bipartite transferrin receptor system (16) (see Table S12 in the supplemental material). Strain 129Pt had three additional, separate ORFs representing C-terminal (HS\_0097), central (HS\_0098), and N-terminal (HS\_0102) fragments of TbpA, which may represent a frameshifted version of the gene encoding the single-component TbpA2 protein of strain 649 (16). In support of this idea, HS\_0097 encodes a 482-amino-acid protein, which corresponded to the last two-thirds of TbpA2 from strain 649, as reported previously (16). We also found a poly(G) tract containing eight G's in HS\_0098; a poly(G) tract present in *Histophilus ovis* strain 3384Y and *H. somnus* strain 649 *tbpA2* genes controls the expression of TbpA2 through phase variation (16, 18). In strain 649 grown under iron-replete conditions, the presence of eight G's in the poly(G) tract introduces a premature stop codon into the reading frame of the gene (16), as do the eight G's in the HS\_0098 fragment. When grown under iron-restricted conditions in the presence of goat transferrin, the poly(G) tract of the strain 649 *tbpA2* gene contains nine G's, removing the frameshift and resulting in a full-length TbpA2 precursor (16). *H. somnus* 129Pt had yet another copy of *tbpA* (HS\_0582) that had 35% amino acid identity to the *H. ovis* and *H. somnus* 649 *tbpA* genes.

In gram-negative bacteria, the TonB system is used for the active transport of iron and heme from host proteins, such as transferrin and hemoglobin, into the periplasmic space (57). The TonB system consists of three essential proteins, TonB, ExbB, and ExbD. *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* Rd had the *tonB*, *exbB*, and *exbD* genes as well as genes encoding outer membrane hemoglobin and heme receptors, many with sequence similarity to TonB-dependent receptors. For example, *H. ducreyi* 35000HP has *hgbA/hupA* (HD2025), which encodes an outer membrane hemoglobin receptor (19), and *tdhA/hemR* (HD0388), encoding a heme receptor, both with amino acid sequence similarity to TonB-dependent outer membrane receptors (19, 66). *H. somnus* 129Pt had *hgbA*, but due to a frameshift, it was split into two ORFs (HS\_0720 and HS\_0721). The *hgbA* genes of *H. somni* ovine strains 9L and 3384Y are also frameshifted, and this finding correlates with an inability of these strains to bind and utilize hemoglobin (67); in the ovine strains 9L and 3384Y, the premature stop codon in *hgbA* occurs much earlier in the sequence (base 642) than in the 129Pt gene (base 2958). The frameshifted *H. somnus* 129Pt *hgbA* gene was adjacent to two genes involved in heme utilization (HS\_0722 and HS\_0723).



Only one of these genes (HS\_0722) had similarity to genes in *H. ducreyi* 35000HP (HD0620) and *H. influenzae* Rd (HI0854). *H. influenzae* Rd had a gene encoding a hemoglobin binding protein with sequence similarity to *H. ducreyi* *hgbA* (HI0661) as well as two copies of *hgbB* (HI0635 and HI0712). *H. somnus* 129Pt and *H. ducreyi* 35000HP did not have *hgbB*. *H. somnus* 129Pt had a gene annotated as *hemR* (HS\_0728), encoding an outer membrane heme receptor with 50% amino acid sequence identity to *H. ducreyi* *tdhA/hemR*; *H. influenzae* Rd also had *hemR* (HI0113). Additional TonB-dependent receptors included *H. somnus* 129Pt gene HS\_0181 and *H. influenzae* Rd genes HI1565 and HI1567. The *H. influenzae* Rd hemoglobin receptor genes *hgbA* (HI0661), *hgbB* (HI0635 and HI0712), and HI1565 have 20 (HI0661 and HI0635), 36 (HI0712), and 18 (HI1565) CAAC repeats close to the gene start that are involved in phase variation of gene expression (32). However, the *H. somnus* 129Pt and *H. ducreyi* 35000HP *hgbA* genes, which were the closest hits to all of the *H. influenzae* Rd hemoglobin receptor genes, did not have these repeats. Therefore, these genes in *H. somnus* 129Pt and *H. ducreyi* 35000HP are unlikely to undergo phase variation. In fact, as the *hgbA* gene in *H. somnus* 129Pt was split into two ORFs (HS\_0720 and HS\_0721), it is probably nonfunctional.

Following the transfer of extracellular protein-bound iron into the periplasm by TonB-dependent receptors, an iron transporter moves iron from the periplasmic space to the cytoplasm (reviewed in references 27 and 58). In *H. influenzae*, this activity is encoded by the *hitABC* (*fbpABC*) genes (61). *H. somnus* 129Pt contained genes with similarity to the *H. influenzae* *hitABC* genes, but *H. ducreyi* 35000HP did not (see Table S12 in the supplemental material). In many bacteria, iron utilization is regulated through the *fur* gene product. In *H. influenzae*, the transferrin receptor genes as well as the genes for hemopexin and hemoglobin receptors are repressed by iron, heme, and protoporphyrin IX, probably through the actions of Fur (29). *H. somnus* 129Pt, *H. ducreyi* 35000HP, and *H. influenzae* all had *fur* homologs, and *H. ducreyi* has a functional *fur* system (9). As *H. influenzae* Rd, *H. ducreyi* 35000HP, and *H. somnus* 129Pt had *fur* homologs, we looked for a Fur binding motif consensus sequence (AATTATTATC) (26) in the genomes to see if it was present in the upstream region of genes involved in iron utilization. We found putative Fur binding motifs in the 500-bp upstream region of the *H. somnus* 129Pt genes encoding a TonB-dependent outer membrane receptor (HS\_0181) and *hugX* (HS\_0723), in two *H. influenzae* Rd genes encoding hemoglobin-binding proteins (HI0635 and HI0712), and in the *H. ducreyi* 35000HP genes encoding hemoglobin utilization protein A (HD2025) and heme-binding protein A (HD0215). This suggests that the transcription of these genes may be regulated by Fur.

In conclusion, a comparison of *H. somnus* 129Pt with the genomes of *H. ducreyi* 35000HP and *H. influenzae* Rd revealed similarities and differences in gene content that belie the abilities of these organisms to utilize certain sugars and amino acids as carbon and nitrogen sources, indicating which compounds are likely to be present in their respective environments. Our finding that all three of these organisms had the components of an incomplete, reductive TCA cycle, which is probably used more for the biosynthesis of intermediates than for energy production, provides another clue about common

and unique aspects of their lifestyles. Finally, we found differences in gene content among these organisms that may contribute to host specificity. These findings included differences in the numbers and types of adhesin and outer membrane protein genes as well as hemoglobin and transferrin receptor genes. In particular, the *H. somnus* 129Pt genome contained 12 genes that probably encode autotransporter adhesins; these genes did not have obvious counterparts in *H. ducreyi* 35000HP and *H. influenzae* Rd, so they may well represent novel functions that contribute to the preference of commensal *H. somnus* 129Pt for the bovine urogenital tract environment. In addition, *H. somnus* 129Pt had genes encoding outer membrane proteins P1, P5, and P6 but not P2 and P4; *H. ducreyi* 35000HP and *H. influenzae* Rd had genes encoding all five of these outer membrane proteins. Taken together with the finding that none of these organisms had genes with significant similarity to the adhesin genes of pathogenic *H. influenzae* strains, these results lead to the speculation that differences in numbers and types of adhesin and outer membrane protein genes may contribute to host specificities and niche differences among commensal and pathogenic strains.

The ability to utilize hemoglobin and transferrin may also play a role in the host specificity of *H. somnus* strains (17, 67). For example, bovine *H. somnus* isolates produce hemoglobin receptors that mediate the uptake of iron from bovine hemoglobin but not from ovine, porcine, or human hemoglobin (67). There is evidence that the ability of *H. somnus* isolates to cause infection in ruminants may depend on the host specificity of their transferrin receptors (74). In particular, *H. somnus* strains that contain single-component transferrin receptors encoded by the *tbpA2* gene can acquire iron from bovine, ovine, and goat transferrins (16), but those that possess bipartite transferrin receptors encoded by the *tbpA* and *tbpB* genes can acquire iron only from bovine transferrin (16, 74). Phase variation of hemoglobin and transferrin receptor genes has been demonstrated in virulent *H. somnus* strains 2336 and 649 (16, 18, 67). Taken together with our finding that commensal strain 129Pt contains truncated hemoglobin (*hgbA*) and single-component transferrin receptor (*tbpA2*) genes, these results support the idea that virulence may depend in part on the ability to utilize iron from hemoglobin and transferrin.

Whole-genome comparisons of *H. somnus* 129Pt with pathogenic isolates will allow us to explore the potential roles of adhesins, hemoglobin and transferrin receptors, and other virulence factors, such as LOS, in host colonization, host specificity, and pathogenesis. As cell surface adhesins and outer membrane proteins are good targets for vaccine development, results of this and future studies will aid in the development of vaccines and other protective strategies against *H. somnus* disease.

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