Genome sequence of *Helicobacter suis* supports its role in gastric pathology

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**Abstract**

*Helicobacter* (*H.*) *suis* has been associated with chronic gastritis and ulcers of the pars oesophagea in pigs, and with gastritis, peptic ulcer disease and gastric mucosa-associated lymphoid tissue lymphoma in humans. In order to obtain better insight into the genes involved in pathogenicity and in the specific adaptation to the gastric environment of *H. suis*, a genome analysis was performed of two *H. suis* strains isolated from the gastric mucosa of swine. Homologs of the vast majority of genes shown to be important for gastric colonization of the human pathogen *H. pylori* were detected in the *H. suis* genome.

*H. suis* encodes several putative outer membrane proteins, of which two similar to the *H. pylori* adhesins HpaA and HorB. *H. suis* harbours an almost complete *comB* type IV secretion system and members of the type IV secretion system 3, but lacks most of the genes present in the *cag* pathogenicity island of *H. pylori*. Homologs of genes encoding the *H. pylori* neutrophil-activating protein and γ-glutamyl transpeptidase were identified in *H. suis*. *H. suis* also possesses several other presumptive virulence-associated genes, including homologs for *mviN*, the *H. pylori* flavodoxin gene, and a homolog of the *H. pylori* vacuolating cytotoxin A gene. It was concluded that although genes coding for some important virulence factors in *H. pylori*, such as the cytotoxin-associated protein (CagA), are not detected in the *H. suis* genome, homologs of other genes associated with colonization and virulence of *H. pylori* and other bacteria are present.

**Introduction**

*Helicobacter* (*H.*) *suis* is a very fastidious, spiral-shaped, Gram-negative bacterium requiring a biphasic culture medium at pH 5 enriched with fetal calf serum, and a microaerobic atmosphere for in vitro growth [1]. *H. suis* colonizes the stomach of more than 60% of slaughter pigs [1,2]. Although the exact role of *H. suis* in gastric disease in pigs is still unclear, it has been associated with chronic gastritis [3,4] and ulcers of the pars oesophagea of the stomach [5-7]. This may result in significant economic losses due to sudden death, decreased feed intake and reduced daily weight gain [8]. A reduction of approximately 20 g/day in weight gain was observed in animals experimentally infected with *H. suis*, compared to the non-infected control animals [9].

Bacterial gastric disorders in humans are mainly caused by *Helicobacter pylori* [10]. However, non-*Helicobacter pylori* helicobacters (NPHH) have also been associated with human gastric disease with a prevalence ranging between 0.2 and 6% [5]. *H. suis* is the most frequent NPHH species found in humans, where it was originally named “*H. helmanii*” type 1 [11]. There are strong indications that pigs may serve as a source of infection for humans [5,12]. In the human host, *H. suis* has been associated with peptic ulcer disease [13], gastric mucosa-associated lymphoid tissue (MALT) lymphoma [14] and chronic gastritis [15]. In rodent models of human gastric disease, the bacterium causes severe inflammation and MALT lymphoma-like lesions [16].

Up to now, little is known about the pathogenesis of *H. suis* infections. To improve understanding in the genes playing a role in pathogenicity, gastric colonization and persistence of *H. suis*, a genome-wide comparison with the well-investigated *H. pylori* genome was performed. Some virulence factors may indeed be similar for both bacteria. As there may also be differences, ab initio annotations of the *H. suis* genome were performed as well.
Materials and methods

Genome sequencing

A pyrosequencing (454 Life Sciences Corporation, Branford, CT, USA) assay was applied to the genome of the type strain of *H. suis* (HS1T = LMG 23995T = DSM 19735T) and *H. suis* strain 5 (HS5), isolated from the gastric mucosa of two different swine, according to the method described by Baele et al. [1]. Quality filtered sequences were assembled into contigs using a 454 Newbler assembler (Roche, Branford, CT, USA).

Functional annotation

In order to maximize the number of quality gene annotations, two different annotating approaches were followed: cross-mapping with three *Helicobacter pylori* strains (26695, Shi470, and G27 with NCBI accession numbers NC_000915, NC_010698, and NC_011333, respectively), and ab initio annotation.

Cross-mapping annotation

A custom BLAST [17] database was created from the HS1T and HS5 genomic contigs. The *H. pylori* proteome and non-coding RNAs were aligned (tblastn program of the BLAST suite, e threshold set to $10^{-3}$) to the *H. suis* database. For each BLAST hit the following additional information was analysed: 1) (secretion) signal peptide cleavage site if present, as assessed by the SignalP 3.0 program [18,19]; 2) specifications of transmembrane helices (number, start and end positions, presumed topology with regard to the cytoplasmic membrane) if present, as assessed by the TMHMM program [20]; 3) an estimate of the ribosome binding strength of the mRNA region preceding the most probable start codon. Ribosome binding strength was estimated by applying two established facts: i) on an mRNA strand, usually within 20 nucleotides before the actual start codon, the reverse complement of 5 to 7 nucleotides near the 16S rRNA 3’ end acts as an attractor and positioner for the ribosomal small subunit; this region is known as the Shine-Dalgarno sequence [21,22]; ii) in Gram-negative bacteria an AU-rich mRNA region some 16 nucleotides long and immediately preceding the Shine-Dalgarno sequence may also attract and position ribosomes to help initiate translation of the correct, biologically active gene product [23,24]. For *H. suis*, the Shine-Dalgarno sequence was determined to be a subsequence of AGGAGGU (which is the reverse complement of the 3’ end of the 16S rRNA), and the minimum AU-richness (equivalent to ribosome binding capacity) of the preceding region was arbitrarily set to 10/16. For each theoretical ORF a range of possible start codons was scored; the higher the similarity to the ideal Shine-Dalgarno sequence, or the AU-richer the preceding region, or the better a combination of both, the more likely the potential start codon is to be the actual start codon.

Ab initio annotation

For ab initio annotation, theoretical open reading frames (ORFs) were first determined using the EMBOSS getorf tool (with minimum ORF length set to 90 nucleotides, and taking all alternative start codons into account) [25]. All ORFs were translated subsequently, and BLAST (blastp program) was performed with an e threshold of $10^{-15}$ against the Uniprot-KB universal protein database. The generalist algorithm of getorf yielded roughly a tenfold of the expected natural ORFs, reducing the risk of false negatives. In order to keep the false positive rate low, extra parameters were considered: 1) percentage alignment between query and hit ORFs; 2) percentage similarity or conservation between aligned portions of query and hit ORFs; 3) ribosome binding strength (for more details see above). To determine the presence of one or more conserved domains a rpstblast search (with default parameter values) was carried out for every single theoretical ORF against the compiled Conserved Domain Database which holds protein domain alignments from several other database sources [26].

Results

General features of the *H. suis* genome

In the HS1T genome a total of 1 635 292 base pairs and in the HS5 genome 1 669 960 bp were sequenced, both with an average GC content of 40%. In contrast to *H. pylori*, only one copy of both the 16S and 23S rRNA genes was detected, but like *H. pylori*, *H. suis* has three copies of the 5S rRNA gene. Thirty-eight transfer RNAs were identified. On the whole, 1266 ORFs from HS1T and 1257 from HS5 were detected, of which 194 and 191 encoded hypothetical proteins respectively. In 98 and 92 ORF a signal peptide cleavage site was detected, demonstrating predicted secreted proteins of HS1T and HS5 respectively. The TMHMM program predicted 210 and 206 proteins with at least one transmembrane helix for HS1T and HS5 respectively. The sequence fraction identical for HS1T and HS5 is henceforward described together as the “*H. suis* genome”.

Genes possibly involved in gastric colonization and persistence

Homologs of *H. pylori* genes involved in acid acclimation, chemotaxis, adhesion to gastric epithelial cells, oxidative stress resistance (Table 1), and motility were detected in the *H. suis* genome. The latter were identified as a flagellar biosystem similar to that of *H. pylori* [27]. Moreover, *H. suis* contains a fibrinonecin/fibrinogen-binding protein coding gene, but the corresponding protein lacks a transmembrane helix or signal peptide cleavage site according to the bioinformatics tools mentioned earlier. Homologs coding for CMP-N-acetylneuraminic acid synthetase (NeuA) (HSUHS1_0474, HSUHS5_0481),
### Table 1 Genes associated with pH homeostasis, chemotaxis, adhesion to epithelial cells, and oxidative stress resistance in the genome of *H. suis* type strain 1 (HS1) and *H. suis* strain 5 (HS5)

| Group                  | Gene detected in HS1^1 | Gene detected in HS5 | Description of homolog | Percentage of sequence aligned (of which % conserved) with described homolog^1 |
|------------------------|------------------------|----------------------|------------------------|--------------------------------------------------------------------------------|
| **pH homeostasis**     | HSUH1S_0708            | HSUH5S_0286          | Urease subunit alfa (*ureA*) of *H. helminthi* | 100 (94)                                                                        |
|                        | HSUH1S_0707            | HSUH5S_0285          | Urease subunit beta (*ureB*) of *H. helminthi* | 100 (94)                                                                        |
|                        | HSUH1S_0706            | HSUH5S_0284          | Urease transporter (*ureD*) of *H. felis*     | 100 (89)                                                                        |
|                        | HSUH1S_0705            | HSUH5S_0283          | Urease accessory protein (*ureE*) of *H. bizzozeronnii* | 100 (84)                                                                        |
|                        | HSUH1S_0704            | HSUH5S_0282          | Urease accessory protein (*ureF*) of *H. bizzozeronnii* | 100 (84)                                                                        |
|                        | HSUH1S_0702            | HSUH5S_0280          | Urease accessory protein (*ureG*) of *H. bizzozeronnii* | 96 (84)                                                                         |
|                        | HSUH1S_0703            | HSUH5S_0281          | Urease accessory protein (*ureH*) of *H. bizzozeronnii* | 100 (95)                                                                        |
|                        | HSUH1S_0133            | HSUH5S_0547          | Hydrogenase expression/formation protein (*hypA*) of *H. pylori* | 98 (83)                                                                         |
|                        | HSUH1S_0615            | HSUH5S_0817          | Hydrogenase expression/formation protein (*hypB*) of *H. pylori* | 99 (91)                                                                         |
|                        | HSUH1S_0616            | HSUH5S_0816          | Hydrogenase expression/formation protein (*hypC*) of *H. pylori* | 98 (89)                                                                         |
|                        | HSUH1S_0617            | HSUH5S_0815          | Hydrogenase expression/formation protein (*hypD*) of *H. achinonychis* | 98 (80)                                                                         |
|                        | HSUH1S_0081            | HSUH5S_1197          | I-Asparaginase II (*amiB*) of *H. pylori*      | 98 (64)                                                                         |
|                        | HSUH1S_0230            | HSUH5S_1130          | Arginase (*rocF*) of *H. pylori*               | 99 (75)                                                                         |
|                        | HSUH1S_0888            | HSUH5S_0231          | Acylamide amidohydrolase (*amiD*) of *H. pylori* | 100 (93)                                                                        |
|                        | HSUH1S_0680            | HSUH5S_0265          | Formamidase (*amiF*) of *H. pylori*            | 100 (98)                                                                        |
|                        | HSUH1S_0161            | HSUH5S_1077          | α-Carboxylic anhydrase of *H. pylori*          | 92 (69)                                                                         |
|                        | HSUH1S_0391            | HSUH5S_0874          | Aspartase (*aspA*) of *H. acinonychis*         | 100 (89)                                                                        |
| Chemotaxis             | HSUH1S_1004            | HSUH5S_0649          | CheA-MCP interaction modulator of *H. pylori*  | 99 (79)                                                                         |
|                        | HSUH1S_1003            | -                    | Bifunctional chemotaxis protein (*chef*) of *H. pylori* | 82 (86)                                                                         |
|                        | HSUH1S_1002            | HSUH5S_0775          | Purine-binding chemotaxis protein (*cheW*) of *H. pylori* | 98 (91)                                                                         |
|                        | HSUH1S_0538            | HSUH5S_0706          | Chemotaxis protein (*cheV*) of *H. pylori*     | 100 (92)                                                                        |
|                        | HSUH1S_0846            | HSUH5S_0081          | Putative chemotaxis protein of *H. pylori*     | 100 (79)                                                                        |
|                        | HSUH1S_0391            | HSUH5S_0874          | Chemotaxis protein (*cheY*) of *H. pylori*     | 100 (95)                                                                        |
|                        | HSUH1S_0286            | HSUH5S_0256          | Methyl-accepting chemotaxis protein (*tipA*) of *H. pylori* | 100 (60)                                                                        |
|                        | HSUH1S_0479            | HSUH5S_0476          | Methyl-accepting chemotaxis protein (*tipB*) of *H. pylori* | 98 (63)                                                                         |
|                        | HSUH1S_0196            | HSUH5S_0122          | Methyl-accepting chemotaxis protein of *Campylobacter upsaliensis* | 99 (53)                                                                         |
|                        | HSUH1S_0141            | HSUH5S_0641          | Methyl-accepting chemotaxis protein of *Campylobacter fetus subsp. fetus* | 99 (64)                                                                         |
|                        | HSUH1S_0763            | -                    | Methyl-accepting chemotaxis protein of *Methylibium petroleiphilum* | 83 (52)                                                                         |
|                        | HSUH1S_0944            | HSUH5S_0990          | Methyl-accepting chemotaxis sensory transducer *Mannomonas sp.* | 57 (59)                                                                         |
| Adhesion               | HSUH1S_0666            | HSUH5S_1053          | Outer membrane protein (*horB*) of *H. pylori* | 100 (63)                                                                        |
|                        | HSUH1S_0354            | HSUH5S_0398          | Neuraminylactose-binding hemagglutinin (*hpaA*) of *H. acinonychis* | 94 (77)                                                                         |

^1 Corresponds to the protein of the describing organism.

^2 Data from a different study.
Table 1 Genes associated with pH homeostasis, chemotaxis, adhesion to epithelial cells, and oxidative stress resistance in the genome of *H. suis* type strain 1 (HS1) and *H. suis* strain 5 (HSS) (Continued)

| Oxidative stress resistance | HSUHS1_1147 | HSUHS5_0608 | Catalase (katA) of *H. acnonychis* | 95 (82) |
|----------------------------|-------------|-------------|------------------------------------|--------|
| HSUHS1_0549                | HSUHS5_1206 | Mismatch repair ATPase (mutS) of *H. hepaticus* | 99 (60) |
| HSUHS1_0163                | HSUHS5_0495 | Superoxide dismutase (sodB) of *H. pylori* | 100 (90) |
| HSUHS1_1186                | HSUHS5_0005 | Bacterioferritin co-migratory protein of *H. hepaticus* | 99 (72) |
| HSUHS1_0683                | HSUHS5_0262 | NAD(P)H quinone reductase (mdaB) of Campylobacter fetus subsp. fetus | 97 (68) |
| HSUHS1_0689                | HSUHS5_0268 | Peroxiredoxin of *H. pylori* | 100 (92) |

1 Resulting from tblastn-based cross-mapping of the *H. pylori* proteome to the *H. suis* HS1 and HSS genomes and blastp-based *ab initio* analyses of the translated *H. suis* HS1 and HSS ORFs against the Uniprot-KB universal protein database. Differences between HS1 and HSS homologs ≤ 1%.

2 Lacking in other Helicobacter genomes available at GenBank.

3 Member of the 2-Cys peroxiredoxin superfamility.

sialic acid synthase (NeuB) (HSUHS1_0477, HSUHS5_0478), and UDP-N-acetylgalactosamine-2-epimerase (WecB) (HSUHS1_1107, HSUHS5_0784) were observed as well.

Genes encoding putative outer membrane proteins (OMPs) in relation to *H. pylori* OMPs are presented in Additional file 1 Table S1. Genes coding for members of major *H. pylori* OMP families (Hop, Hor, Hof proteins, iron-regulated and efflux pump OMPs) could be aligned with the *H. pylori* genome. Both *H. suis* strains contain the *hop* genes *hofA*, *C*, *E*, *F*, the *hop* genes *hopE*, *G*-2 and *H*, and the *hor* genes *horB*, *C*, *D*, and *J*. Additionally, HS1 contains homologs of the *hopW* protein precursor and *horE*, whereas HS5 possesses additional homologs of *horA*, *horF*, and *horL*. No members of the *Helicobacter* outer membrane (*hom*) family were detected in *H. suis*. Besides the major *H. pylori* OMP family proteins, the *H. suis* genome contains some predicted OMPs based on their N-terminal pattern of alternating hydrophobic amino acids similar to porins, encompassing *omp29* for HS1 and *omp11* and *omp29* for HSS. A 491 amino acids membrane-associated homolog of the virulence factor MviN, aligned for 92% with the MviN homolog of *H. acnonychis* (Hac_1250), was also present in *H. suis*.

Type IV secretion systems in *H. suis*

Of the *H. pylori* type IV secretion systems (T4SS), only two members of the *cag* pathogenicity island (*cagPAI*) were identified in the *H. suis* genome (*cag23/E* and *cagX*). Most members of the *comB* transport apparatus were present. These include *comB2*, *B3*, *B6*, *B8* and a number of additional genes not classified as *comB*: *recA*, *comE*, *comL* and *dprA*. *H. suis* possesses genes encoding VirB- and VirD-type ATPases (*virB4*, *B8*, *B9*, *B10*, *B11*, and *virD2*, *D4*), all designated members of the *H. pylori* type IV secretion system 3 (fts3). The HS1 and HSS T4SS are presented in Table 2.

Genes possibly involved in induction of gastric lesions

Homologs of *H. pylori* genes involved in induction of gastric lesions in the *H. suis* genome are summarized in Table 3. Homology searches with the *H. pylori* vacuolating cytotoxin A gene (*vacA*) identified HSUHS1_0989 in HS1. The corresponding protein, which is exceptional in that it is one of the longest in the world of prokaryotes, possesses three small conserved VacA regions (residues 490-545, 941-995, and 1043-1351), followed by an auto-transporter region (residues 2730-2983). The amino acid sequence of the HS5 homolog (HSUHS5_0761) could be aligned for 22% with the *H. pylori* strain HPAG1 sequence, and possesses only one conserved VacA region (residues 242-298), followed by an autotransporter region (1258-1510). In both vacA homologs, no signal sequence was determined. Additionally, an ulcer-associated adenine-specific DNA methyltransferase (HSUHS1_0375, HSUHS5_0957) coding sequence was identified, whereas a molecular homolog of the ulcer-associated restriction endonuclease (*iceA*) could not be discovered in *H. suis*. *H. suis* contains homologs of *pgbA* and *pgbB* encoding plasminogen-binding proteins, though both lacking a transmembrane helix or signal peptide cleavage site according to the bioinformatics tools mentioned earlier.

*H. suis* harbours homologs of genes coding for the *H. pylori* neutrophil-activating protein (HP-NapA) and γ-glutamyl transpeptidase (HP-GGT). Homologs encoding the *H. pylori* flavodoxin *fldA* and the pyruvate-oxidoreductase complex (POR) members *porA*, *porB*, *porC*, and *porD* were also identified in *H. suis*.

Discussion

Genes possibly involved in gastric colonization and persistence

The results of the present study demonstrate that several *H. pylori* genes involved in acid acclimation, chemotaxis and motility, have counterparts in the *H. suis*
genome. These genes are known to be essential for colonization of the human gastric mucosa [27-32]. Several OMP coding sequences were identified by comparative analyses with H. pylori and other bacterial species. H. suis contains some similar members of the major OMP families described in H. pylori [33]. Some of these OMPs have been described to be involved in adhesion of H. pylori to the gastric mucosa, which is widely assumed to play an important role in the initial colonization and long-term persistence in the human stomach. These include the gastric epithelial cell adhesin HrbB [34] and the surface lipoprotein, H. pylori adhesin A (HpaA). HpaA, also annotated as neuraminylactose-binding hemagglutinin, is found exclusively in Helicobacter and binds to sialic acid-rich macromolecules present on the gastric epithelium [35]. On the other hand, H. suis lacks homologs of several other H. pylori adhesion factors, including genes coding for the blood group antigen binding adhesins babA (hopS) and babB (hopT), the sialic acid binding adhesins sabA (hopP) and sabB (hopO), and the adherence-associated lipoproteins alpA (hopC) and alpB (hopB) [36].

H. suis contains a fibrinonectin/fibrinogen-binding protein coding gene, which may enhance its adherence to injured gastric tissue. Damage to host epithelial cells may indeed expose fibronectin and other extracellular protein detected in H55.

| Homolog       | Gene detected in H5T | Gene detected in H55 | Description of corresponding protein | Percentage of sequence fraction aligned (of which % conserved) with Helicobacter homolog |
|---------------|----------------------|----------------------|--------------------------------------|---------------------------------------------|
| cog pathogenicity island |                       |                      |                                      |                                             |
| cag23/E of H. pylori | HSUHS1_0731           | HSUHSS_1234          | DNA transfer protein                 | 81 (42)                                     |
| cagX of H. pylori   | HSUHS1_0964           | HSUHSS_0688          | Conjugal plasmid transfer protein    | 92 (71)                                     |
| comB system       |                       |                      |                                      |                                             |
| comB2 of H. acinonychis | HSUHS1_1181         | HSUHSS_0010          | ComB2 protein                        | 96 (64)                                     |
| comB3 of H. acinonychis | HSUHS1_1182         | HSUHSS_0009          | ComB3 competence protein             | 95 (77)                                     |
| comB6 of H. pylori | HSUHS1_0337           | -                    | NADH-ubiquinone oxidoreductase       | 70 (85)                                     |
| comB8 of H. pylori | HSUHS1_0747           | -                    | Overlap with virB8                   | 93 (66)                                     |
| comE of H. acinonychis | HSUHS1_0314         | HSUHSS_0381          | Competence locus E                   | 94 (55)                                     |
| comL of H. pylori  | HSUHS1_0722           | HSUHSS_0300          | Competence protein                   | 99 (84)                                     |
| dprA of H. acinonychis | HSUHS1_0096         | HSUHSS_0824          | DNA processing protein               | 99 (70)                                     |
| recA of H. hepaticus | HSUHS1_0672          | HSUHSS_1058          | Recombinase A                        | 97 (84)                                     |
| virB - homologs    |                       |                      |                                      |                                             |
| virB4 of H. pylori | HSUHS1_0960           | HSUHSS_0692          | DNA transfer protein                 | 98 (68)                                     |
| virB8 of H. pylori | HSUHS1_0963           | HSUHSS_0689          | DNA transfer protein                 | 91 (61)                                     |
| virB9 of H. cetorum | HSUHS1_0319          | -                    | VirB9 protein                        | 76 (69)                                     |
| virB10 of H. cerorum | HSUHS1_0320          | -                    | VirB10 protein                       | 90 (77)                                     |
| putative virB9 of H. pylori | -               | HSUHSS_0372          | Putative VirB9 protein               | 100 (86)                                    |
| putative virB10 of H. pylori | -              | HSUHSS_0371          | Putative VirB10 protein              | 97 (87)                                     |
| virB11 of H. pylori | HSUHS1_0750           | HSUHSS_0368          | VirB11 protein                       | 100 (98)                                    |
| virB11 of H. cetorum | HSUHS1_0965          | -                    | VirB11 protein                       | 95 (71)                                     |
| virB11-like of H. pylori (HPSH_04565) | -            | HSUHSS_0686          | VirB11-like protein                  | 98 (72)                                     |
| virB11-like of H. pylori (HPSH_07250) | HSUHS1_0036        | HSUHSS_0600          | Type I ATPase                        | 100 (75)                                    |
| virD - homologs    |                       |                      |                                      |                                             |
| virD2 of H. cetorum | HSUHS1_0752           | HSUHSS_0414          | VirD2 protein (relaxase)             | 100 (90)                                    |
| virD4 of H. pylori  | HSUHS1_0870           | HSUHSS_0257          | VirD4 protein (conjugation protein)  | 82 (78)                                     |

1Resulting from tblastn-based cross-mapping of the H. pylori proteome to the H. suis H5T and H55 genomes and blastp-based ab initio analyses of the translated H. suis H5T and H55 ORFs against the Uniprot-KB universal protein database. Differences between H5T and H55 homologs ≤ 1%.
Two partial T4SS were predicted in the *H. suis* genome, namely the **comB** cluster and the **tfs3** system. The *H. suis** comB system probably plays a role in genetic transformation [41,42]. Transformation of DNA can be responsible for the high degree of diversity among *H. suis* strains as has been recently demonstrated by multilocus sequence typing of available *H. suis* strains [43]. The role of the *H. pylori* **tfs3** secretion system in pathogenesis is not exactly known. Seven genes of the **tfs3** cluster are homologs of genes involved in type IV secretion: **virB4**, **virB11**, and **virD4** code for ATPases which move substrates to and through the pore. The latter is coded by transmembrane pore genes **virB7**, **virB8**, **virB9**, and **virB10** [44]. All these genes, except **virB7** were identified in *H. suis*, indicating that the *H. suis* **tfs3** can be important in transmembrane transport of substrates in *H. suis*. The *H. pylori* **cag** pathogenicity island (**cag**PAI) region encodes a T4SS allowing *H. pylori* to insert the cytotoxin-associated antigen A (CagA) into the host cell. This process results in altered host cell structure, an increased inflammatory response, and a higher risk for gastric adenocarcinoma [45]. Although *H. suis* possesses two members of the *H. pylori* **cag**PAI (**cag23/E** and **cagX**), the majority of genes, including the gene coding for pathology-causing protein (CagA), were not identified. This indicates that **HS1** and HS5 lack a functional **cag** protein transporter secretion system.

**Table 3 Homologs of *H. pylori* genes involved in induction of gastric lesions in the *H. suis* type strain 1 (HS1<sup>1</sup>) and strain 5 (HS5) genome**

| Gene detected in HS1<sup>1</sup> | Gene detected in HS5 | Gene name | Protein annotation/function in *H. pylori* | Sequence fraction HS1<sup>1</sup>/HS5 aligned with *H. pylori* homolog (%)<sup>1</sup> | Aligned sequence fraction HS1<sup>1</sup>/HS5 conserved with *H. pylori* homolog (%)<sup>1</sup> | References |
|---------------------------------|---------------------|-----------|----------------------------------------|---------------------------------|---------------------------------|-------------|
| HSUHS1_0989                      | HSUHSS_0761         | vacA      | Vacuolating cytotoxin A: host cell vacuolation, apoptosis-inducing, immunosuppressive | 63/22                           | 45/72                           | [46]        |
| HSUHS1_0265                      | HSUHSS_0449         | ggt       | γ-glutamyl transpeptidase: apoptosis-inducing, immunosuppressive | 99/99                           | 86/86                           | [48,49,64] |
| HSUHS1_1177                      | HSUHSS_0014         | napA      | Neutrophil-activating protein A: proinflammatory | 99/99                           | 83/83                           | [50,51]     |
| HSUHS1_1067                      | HSUHSS_1177         | fdiA      | Electron acceptor of the pyruvate oxidoreductase enzyme complex, associated with gastric MALT lymphoma in humans | 96/98                           | 84/83                           | [55,56]     |
| HSUHS1_0403                      | HSUHSS_0887         | pgbA      | Plasminogen-binding protein              | 60/60                           | 72/72                           | [53,54]     |
| HSUHS1_1192                      | HSUHSS_0523         | pgbB      | Plasminogen-binding protein              | 70/70                           | 72/72                           | [53,54]     |

<sup>1</sup>Resulting from tblastn-based cross-mapping of the *H. pylori* proteome to the *H. suis* HS1<sup>1</sup> and HS5 genomes.

matrix components. Strong homology was found with fibronectin-binding proteins of *H. felis* (YP_004072974), *H. canadensis* (ZIP_048703091) and *Wolinella succinogentens* (NP_907753). To our knowledge, no exact function has been given to these proteins in these species. In *Campylobacter jejuni*, however, fibronectin-binding proteins CadF and FlpA have been shown to be involved in adherence to and/or invasion of host's intestinal epithelial cells [37,38]. According to the bioinformatics tools used here, the *H. suis* fibronectin-binding protein lacks a transmembrane helix or signal peptidase cleavage site, indicating that it is not surface exposed or secreted. Its real role in colonization therefore remains to be elucidated.

Three genes involved in sialic acid biosynthesis (**neuA**, **neuB**, and **wecB**) were annotated in the *H. suis* genome, indicating that this bacterium may decorate its surface with sialic acid. The presence of surface sialylation has been studied extensively in pathogenic bacteria, where it is used here, the *H. suis* (**tfs3** cluster and the **virB** system probably plays a role in genetic transformation [41,42]. Transformation of DNA can be responsible for the high degree of diversity among *H. suis* strains as has been recently demonstrated by multilocus sequence typing of available *H. suis* strains [43]. The role of the *H. pylori** tfs3** secretion system in pathogenesis is not exactly known. Seven genes of the **tfs3** cluster are homologs of genes involved in type IV secretion: **virB4**, **virB11**, and **virD4** code for ATPases which move substrates to and through the pore. The latter is coded by transmembrane pore genes **virB7**, **virB8**, **virB9**, and **virB10** [44]. All these genes, except **virB7** were identified in *H. suis*, indicating that the *H. suis** tfs3** can be important in transmembrane transport of substrates in *H. suis*. The *H. pylori* **cag** pathogenicity island (**cag**PAI) region encodes a T4SS allowing *H. pylori* to insert the cytotoxin-associated antigen A (CagA) into the host cell. This process results in altered host cell structure, an increased inflammatory response, and a higher risk for gastric adenocarcinoma [45]. Although *H. suis* possesses two members of the *H. pylori** cag**PAI (**cag23/E** and **cagX**), the majority of genes, including the gene coding for pathology-causing protein (CagA), were not identified. This indicates that **HS1** and HS5 lack a functional **cag** protein transporter secretion system.

**Genes possibly involved in induction of gastric lesions**

Genomic comparison of *H. suis* with *H. pylori* resulted in the identification of additional genes possibly associated with virulence in *H. suis*. A *H. suis* homolog of the *H. pylori* vacA was detected. VacA is both a cytotoxin of the gastric epithelial cell layer, and an immunomodulatory toxin of *H. pylori* [46]. *H. pylori* contains either a functional or non-functional vacA. The *H. suis* vacA homolog exhibits no vacA signal sequence, indicating
that it might encode a non-functional cytotoxin [47]. In vitro and in vivo studies with a knockout mutant of the 
*H. suis* vacA could clarify the functionality of the vacA homolog in this *Helicobacter* species.

Strong homology was found with two *H. pylori* virulence-associated genes namely napA, encoding the HP-
NapA and ggt, encoding HP-GGT. The *H. pylori* GGT has been identified as an apoptosis-inducing protein
[48,49]. The HP-NapA protein is designated as a proin-
flammatory and immunodominant protein by stimulat-
ing production of oxygen radicals and IL-12 from
neutrophils and recruiting leukocytes in vivo [50,51].
Moreover, HP-NapA also plays a role in protecting
*H. pylori* from oxidative stress by binding free iron [52].
*H. suis* contains homologs of two *H. pylori* genes coding
for plasminogen-binding proteins, pgbA and pgbB. The corresponding proteins, PgbA and PgbB bind host plas-
minogen, which subsequently can be activated to plas-
min and may contribute to obstructing the natural
healing process of gastric ulcers [53,54]. The biological
role of the *H. suis* pgbA and B homologs in chronicity
of gastric ulceration is uncertain, as no exact membrane
association was found in the corresponding proteins.

The risk to develop MALT lymphomas in *H. suis*
infected human patients is higher than after infection
with *H. pylori* [5,14]. Homologs encoding the *H. pylori*
flavodoxin (fldA) and its electron donor, the POR
enzyme complex (porA to D) were found in *H. suis*. The
*H. pylori* flavodoxin protein (Flda) has been proposed
to play a role in the pathogenesis of *H. pylori*-associate-
MALT lymphoma, as antibodies against the *H. pylori*
FldA protein were more prevalent in patients with
MALT lymphomas compared to patients with other
*H. pylori*-related diseases [55]. Besides, insertion muta-
genesis of the fldA and the por complex has shown that
these genes are essential for the survival of *H. pylori*
[56]. These observations indicate that fldA and its por
complex may play a role in gastric colonization of
*H. suis* and MALT lymphoma development in *H. suis*
infected people.

Recently, the genomes of the carcinogenic *H. pylori*
strain B38 and the carcinogenic and ulcerogenic *Helico-
bacter mustelae* have been sequenced [57,58]. Both heli-
cobacters lack homologs of major *H. pylori* virulence
genes (e.g. cagA, babA/B, sabA/B), which are also absent
in the *H. suis* genome. Additionally, *H. mustelae* lacks a
vacA homolog. Despite this absence, infection with
*H. pylori* strain B38 and *H. mustelae* has been associated
with gastric MALT lymphomas and other gastric disor-
ders. Whole genome sequencing data are also available
from *H. acinonychis* strain Sheeba, a gastric pathogen
of large felines. Similar to *H. suis*, *H. acinonychis* lacks a
cagPAI as well as genes encoding BabA/B and SabA/B.
Both species contain a vacA homolog, which for *H. aci-
nonychis* has been described to be fragmented [59,60].

*H. suis* contains a mviN homolog. This gene has been
described to be a virulence factor of several bacterial
species, such as *Burkholderia pseudomallei* and *Vibrio
alginolyticus* [61,62]. In addition to virulence, MviN has
been described to be essential for in vitro growth of
these and other bacteria [61-63]. The biological signifi-
cance of mviN in the *Helicobacter* genus, however,
remains to be elucidated.

**Conclusion**

Although *H. suis* lacks homologs of some major
*H. pylori* virulence genes, other candidate virulence fac-
tors, such as napA, ggt, mviN, and fldA were detected.
*H. suis* also possesses genes known to be essential for
gastric colonization. Future in vitro and in vivo research
of the currently presented genes of this porcine and
human gastric pathogen should elucidate their precise
role in colonization and virulence.

**Nucleotide sequence accession numbers**

The genome sequences have been deposited at
GenBank/EMBL/DDBJ under the accession ADGY00
000000 for HS1 T and ADHO00000000 for HS5. The
versions described in this paper are the first versions,
ADGY1000000 and ADHO1000000.

**Additional material**

Additional file 1: Table S1 Classification of *H. suis* strain 1 (HS1T)
and strain 5 (HS5) outer membrane proteins (OMPs) in relation to
*H. pylori* OMPs. Additional file Table S1 presents the classification of
*H. suis* outer membrane proteins in relation to *H. pylori* outer membrane
proteins. Although this table is not essential, we believe that it is both a
relevant and interesting addition to the content of the article.

**Acknowledgements**

This work was supported by the Research Fund of Ghent University, Belgium (project no. 01G00408), and by the Agency for Innovation by Science and Technology in Flandres (IWT) (grant no. SB-091002). We thank Mrs Sofie De Bruycere and Mrs Marleen Foubert for her technical support.

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**Authors’ contributions**

MV designed the study, analysed the data and drafted the manuscript. TTMV carried out the sequence alignment and participated in the design of the study. BF, AS and DDG participated in the design of the study. FP, WVC, RD, and FH coordinated and participated in the design of the study. All authors read and approved the final manuscript.
Competing interests
The authors declare that they have no competing interests.

Received: 10 December 2010 Accepted: 17 March 2011
Published: 17 March 2011

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