Identification of putative adhesins of *Actinobacillus suis* and their homologues in other members of the family *Pasteurellaceae*

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Abstract

**Background:** *Actinobacillus suis* disease has been reported in a wide range of vertebrate species, but is most commonly found in swine. *A. suis* is a commensal of the tonsils of the soft palate of swine, but in the presence of unknown stimuli it can invade the bloodstream, causing septicaemia and sequelae such as meningitis, arthritis, and death. It is genotypically and phenotypically similar to *A. pleuropneumoniae*, the causative agent of pleuropneumonia, and to other members of the family *Pasteurellaceae* that colonise tonsils. At present, very little is known about the genes involved in attachment, colonisation, and invasion by *A. suis* (or related members of the tonsil microbiota).

**Results:** Bioinformatic analyses of the *A. suis* H91-0380 genome were done using BASys and blastx in GenBank. Forty-seven putative adhesin-associated genes predicted to encode 24 putative adhesins were discovered. Among these are 6 autotransporters, 25 fimbriae-associated genes (encoding 3 adhesins), 12 outer membrane proteins, and 4 additional genes (encoding 3 adhesins). With the exception of 2 autotransporter-encoding genes (*aidA* and *ycgV*), both with described roles in virulence in other species, all of the putative adhesin-associated genes had homologues in *A. pleuropneumoniae*. However, the majority of the closest homologues of the *A. suis* adhesins are found in *A. ureae* and *A. capsulatus*—species not known to infect swine, but both of which can cause systemic infections.

**Conclusions:** *A. suis* and *A. pleuropneumoniae* share many of the same putative adhesins, suggesting that the different diseases, tissue tropism, and host range of these pathogens are due to subtle genetic differences, or perhaps differential expression of virulence factors during infection. However, many of the putative adhesins of *A. suis* share even greater homology with those of other pathogens within the family *Pasteurellaceae*. Similar to *A. suis*, these pathogens (*A. capsulatus* and *A. ureae*) cause systemic infections and it is tempting to speculate that they employ similar strategies to invade the host, but more work is needed before that assertion can be made. This work begins to examine adhesin-associated factors that allow some members of the family *Pasteurellaceae* to invade the bloodstream while others cause a more localised infection.

**Keywords:** *Actinobacillus suis*, *Pasteurellaceae*, Adhesins, Bioinformatics
A. pleuropneumoniae and A. suis cause different diseases in swine, and A. suis has a broader host range [5].

Little is known about the virulence factors of A. suis, particularly the adhesins. Therefore, the objective of this study was to use bioinformatics tools to mine the newly annotated genome of a clinical isolate of A. suis [6] and identify adhesin-associated genes that may be involved in the early stages of pathogenesis of this organism. Adhesins play an important role in the pathogenesis of most bacteria by allowing them to attach to, colonise, and invade their hosts. In addition to host-pathogen interactions, adhesins are also critical in adherence to abiotic surfaces, auto-aggregation to other bacteria, and in the early stages of biofilm formation [7–9]. Adhesins are often classified as either fimbrial or afimbrial, where fimbrial adhesins are composed of multiple copies of one protein assembled into long appendages such as pili, and afimbrial adhesins are single proteins (e.g., autotransporters or outer membrane proteins) that have adhesive properties [10].

In the current study, we have identified proteins belonging to four different classes of adhesin-associated genes present in the A. suis genome (one fimbrial and three afimbrial) and provided a brief summary of their described roles in attachment in other members of the family Pasteurellaceae, with special emphasis on species in the genus Actinobacillus.

Results and discussion

Forty-seven putative adhesin-associated genes predicted to encode 24 adhesins were identified in the A. suis H91-0380 genome. These genes were categorised as autotransporter-encoding (Table 1), fimbriae-associated adhesins (Table 2), outer membrane proteins (OMPs; Table 3), and miscellaneous adhesins (Table 4).

Autotransporters

Six autotransporter-encoding genes were identified in the A. suis genome (Table 1). Among these, 4 encode proteins that belong to the subfamily known as trimeric autotransporter adhesins (TAAs). Autotransporters are large proteins with three domains—an N-terminal signal domain (present in the immature form of the protein, cleaved from the mature protein), a passenger domain, and a C-terminal translocator domain. In the case of TAAs, the translocator domain is short, and the adhesin structure is formed by a homotrimerisation of the encoded protein [11]. Examples of classic TAAs include Hia in Haemophilus influenzae [12] and YadA in Yersinia enterocolitica [13, 14], and they are characterised by a conserved YadA domain and resistance to proteolytic degradation. All TAAs described to date have adhesive properties and bind to different host components including epithelial cells, extracellular matrix components, and circulating molecules (e.g., complement inhibitory proteins, immunoglobulins) [11].

The four genes encoding TAAs identified in the A. suis genome, ASU2_04675, ASU2_06645, ASU2_07040, and ASU2_11275, are all well conserved in A. capsulatus (E value = 0.0). They also have homologues in A. pleuropneumoniae (E values = 0.0–5e–25), but the top homologues are found in different serovars. These TAAs also share homology with genes in distant species (E values ranging from 2e–14 to 2e–90). Given that many of the distant species (e.g., Collimonas, Megaphera, Advenella, Actinetobacter spp.) with homologues of the A. suis-encoded TAAs are environmental isolates, this may hint that these TAAs are well conserved throughout evolution.

The other two autotransporter genes identified in the A. suis genome encode putative conventional autotransporters. These proteins have the same domains as TAAs, but have a longer translocator domain. In addition to being adhesins that play important roles in attachment and biofilm formation, these autotransporters can have additional properties such as cytotoxic, proteolytic or lipolytic activity, and may play a role in serum resistance [11]. In the A. suis genome, the putative conventional autotransporter-encoding genes, ASU2_07665 and ASU2_11100, are annotated as ycgV and aidA, respectively. While the ycgV gene is well conserved in A. capsulatus (E-value = 0.0) and aidA is quite well conserved in A. ureae (E value = 5e–132), there were no close homologues in A. pleuropneumoniae. It is also noteworthy that in a search for motifs in aidA done using Pfam, no conserved motifs, including the hallmark domains of conventional autotransporters, were detected. Therefore, the classification as an autotransporter-encoding gene relied solely on homology to other autotransporter-encoding genes in GenBank and annotation by BASys. The top homologue of aidA identified in species outside the family Pasteurellaceae was in the Gram positive bacterium Streptococcus suis. However, almost all aidA homologues in Streptococcus species are annotated as hypothetical proteins (with the exception of one homologue which is annotated as the LPXTG-motif cell wall anchor domain protein), and the E value (3e–11), coverage (53 %), and identity (31 %) of the top Streptococcus suis homologue suggest that the degree of conservation of this gene is low. The homology of the A. suis aidA gene with species such as streptococci that share a common environment in the upper respiratory tract of swine may hint at convergent evolution or horizontal gene transfer, but further studies would have to be done to rigorously test such assertions.
Fimbriae-associated adhesins

Twenty-five putative fimbriae-associated adhesin genes were also identified (Table 2). These included 14 genes predicted to be part of a tight adherence (tad) locus, a type IV pilus operon (4 genes), another type IV pilus biogenesis locus containing 6 genes, and another pilus-associated gene.

The pilF gene (ASU2_00450) annotated as a putative fimbrial biogenesis and twitching motility protein, is well conserved in Pasteurellaceae. It is less well conserved outside the family, but pilF homologues are present in Pseudomonas aeruginosa and in Neisseria meningitidis (pilW) and are thought to encode a protein that is critical for pilus stability and function, including attachment to human cells [15, 16]. Pfam analysis revealed TPR repeats in the A. suis pilF gene. In other species, these repeats are thought to play a role in protein–protein interactions in both prokaryotic and eukaryotic cells, and contribute to virulence of bacterial pathogens by aiding in attachment to and invasion of host cells and circumventing host defences [17].

The tad locus is a conserved widespread colonisation island [18] that plays an important role in pathogenesis, biofilm formation, and colonisation of several organisms, including members of the family Pasteurellaceae [19–22]. The tad locus encodes the machinery needed to assemble the fimbrial low-molecular-weight protein (Flp) pilin into long, bundled type IVb pili [21, 22]. The A. suis genome contains a tad locus comprised of homologues of flp1-flp2-tadV-rcpC-rcpA-rcpB-tadZ-tadA-tadB-tadC-tadD-tadE-tadF-tadG. The two putative pilin genes, ASU2_04295 and ASU2_04300, are predicted to encode flp1 and flp2, respectively; however, it may be noted that flp2 is not expressed in Aggregatibacter actinomycetemcomitans [22]. Neither of these genes is very highly conserved within the family Pasteurellaceae and is even less so in more distant species. This may reflect the fact that the flp1 and flp2 putative pilin genes in A. suis have adapted for colonisation of different hosts or different host cell receptors. A genetic analysis of the tad locus by Li et al. [23] revealed that flp1 is truncated or missing altogether in some strains of A. pleuropneumoniae. In the same study, these authors found that tadC is the best conserved among A. pleuropneumoniae strains tested and tadG the least, findings that were not observed in this work when the same genes in the A. suis genome were compared to other species. However, many of the biogenesis components of the tad locus of A. suis are well conserved in A. pleuropneumoniae and other members of the family Pasteurellaceae such as A. capsulatus, and much less well conserved outside the family.

In addition to the tad locus, the A. suis genome also has two other loci for type IV pilus biogenesis: a type IV pilus locus (pilABCD/apfABCD) and a homologue of

| Table 1 Putative autotransporter-encoding genes |
|-----------------------------------------------|
| **ASU2 locus tag** | **GenInfo (GI) number** | **(Possible) gene name** | **GenBank Annotated protein function** | **Top App homologue/E value** | **Top Pasteurellaceae homologue/E value** | **Top other homologue/E value** |
| ASU2_04675 | 407388580 | –a | Autotransporter adhesin autotransporter adhesin | Ser. 10 str. D13039 2e−124 | Actinobacillus capsulatus 0.0 | Collimonas fungi 1e−54 |
| ASU2_06645 | 407388974 | –a | Autotransporter adhesin autotransporter adhesin | Ser. 2 str. 4226 0.0 | Actinobacillus capsulatus 0.0 | Megasphaera genomosp. Type 1 2e−90 |
| ASU2_07040 | 407389053 | –a | Extracellular matrix protein adhesin A extracellular matrix protein adhesin A | Ser. 13 str. N273 5e−25 | Actinobacillus capsulatus 0.0 | Advenella mimi-gardefordensis DPN7 2e−14 |
| ASU2_07665 | 407389178 | ycglV (tibA)bc | Outer membrane autotransporter | outer membrane: Ser. 2 str. S1536 1.3 | Actinobacillus capsulatus 0.0 | Snodgrassella alvi 2e−37 |
| ASU2_11100 | 407389854 | aidAabc | Putative pertactin Ser. 7 str. AP76 | family virulence factor, OM autotransporter/Type V secretory pathway, adhesin | 5e−132 | Actinobacillus ureae 5e−132 |
| ASU2_11275 | 407389889 | –a | Autotransporter adhesin Autotransporter adhesin | Ser. 6 str. Femo 2e−58 | Actinobacillus capsulatus 0.0 | Acinetobacter sp. 5e−65 |

(*) indicates a suggested name that was not present in the annotation
a Function assigned by conserved motifs
b Identified by BASys
c Classified by description of homologues
### Table 2 Putative fimbriae-associated genes

| ASU2 locus tag | GenInfo (GI) number | (Possible) gene name | Annotated protein function | Top App homologue/ E value | Top Pasteurellaceae homologue/E value | Top other homologue/E value |
|----------------|---------------------|----------------------|---------------------------|---------------------------|--------------------------------------|-----------------------------|
| ASU2_00450     | 407387739           | (pfIF)               | Putative fimbrial biogenesis and twitching motility protein PilF-like protein | Ser. 3 str. JL03 2E—116 | Actinobacillus capsulatus 4e—114 | Vibrio nigripulchritudo 3e—28 |
| ASU2_04295     | 407388504           | flp1                 | flp operon protein         | Ser. 4 str. M62 1e—24   | Actinobacillus capsulatus 2E—27     | Vibrio owensii 2e—05        |
| ASU2_04300     | 407388505           | (flp2)               | Hypothetical protein       | Set. 7 str. AP76 1e—16   | Actinobacillus ureae 3E—24          | Vibrio sp. HENC-02 4.3      |
| ASU2_04305     | 407388506           | (tadV)               | flp operon protein; Flp pilus assembly protein, protease CpaA | Ser. 5b str. L20 9E—69  | Actinobacillus capsulatus 6e—55     | Yersinia rohdei ATCC 43380 5e—23 |
| ASU2_04310     | 407388507           | (rcpC)               | flp operon protein C       | Ser. 4 str. M62 3e—123   | Actinobacillus capsulatus 3E—164    | Yersinia similis 2e—19       |
| ASU2_04315     | 407388508           | (rcpA)               | Rough colony protein A; Flp pilus assembly protein, secretin CpaC | Ser. 7 str. AP76 0.0   | Actinobacillus capsulatus 0.0        | Yersinia similis 1e—101      |
| ASU2_04320     | 407388509           | rcpB                 | Rough colony protein B     | Ser. 12 str. 1096 4e—84  | Actinobacillus capsulatus 1E—102    | Ochotona princeps 2.3        |
| ASU2_04325     | 407388510           | (tadZ)               | flp pilus assembly protein, ATPase; CpaE | Ser. 5b str. L20 0.0   | Actinobacillus capsulatus 0.0        | Yersinia berovieri 49790 3e—58 |
| ASU2_04330     | 407388511           | tadA                 | Tight adherence protein A; Flp pilus assembly protein, ATPase CpaF | Ser. 5b str. L20 0.0 | Actinobacillus capsulatus 0.0        | Yersinia aldovae ATCC 35236 4e—179 |
| ASU2_04335     | 407388512           | tadB                 | Tight adherence protein B; Flp pilus assembly protein TadB | Ser. 1 str. 4074 0.0   | Actinobacillus capsulatus 0.0        | Serratia marcescens VGH1107 5e—64 |
| ASU2_04340     | 407388513           | tadC                 | Tight adherence protein C; Flp pilus assembly protein TadC | Ser. 13 str. N-273 8e—160 | Actinobacillus capsulatus 6E—164 | Serratia marcescens VGH1107 9e—36 |
| ASU2_04345     | 407388514           | tadD                 | Tight adherence protein D; Flp pilus assembly protein TadD, contains TPR repeats | Ser. 13 str. N-273 3e—129 | Actinobacillus capsulatus 8E—148 | Hafnia alvei ATCC S1873 7e—52 |
| ASU2_04350     | 407388515           | tadE                 | Tight adherence protein E  | Ser. 10 str. D13039 2E—86 | Actinobacillus capsulatus 3e—84 | Serratia marcescens VGH1107 2e—25 |
| ASU2_04355     | 407388516           | tadF                 | Tight adherence protein F  | Ser. 6 str. Femo 2E—66  | Actinobacillus capsulatus 1E—59 | Yersinia enterocolitica 2e—12 |
| ASU2_04360     | 407388517           | tadG                 | Tight adherence protein G  | Ser. 2 str. S1536 0.0   | Mannheimia haemolytica 0.0           | Yersinia frederiksenii ATCC 33641 6e—19 |
| ASU2_05030     | 407388651           | (polD)               | Fimbrial leader peptidase, Type II secretion pathway, prepilin signal peptidase PulO and related peptidases | Ser. 1 str. 4074 3e—56 | Actinobacillus ureae 1E—135 | Enterococcus faecium EnGen0131 0.014 |
the comABCDEF locus. Type IV pili are important virulence factors in many Gram negative organisms, including other members of the family Pasteurellaceae such as nontypeable Haemophilus influenzae (NTHi) [24–27], Pasteurella multocida [28], and A. pleuropneumoniae [29–31]. In these species, type IV pili have demonstrated roles in biofilm formation, attachment to epithelial cells, twitching motility, competence, and interactions with phage [32–34]. In A. pleuropneumoniae, the apfA pilin gene is present in all strains and is well conserved in all serovars [31]. The homologue of this gene in A. suis (ASU2_05045) is the least well conserved gene in the pilABCD locus, but is still homologous to genes in both A. uraeae (1e−70) and A. pleuropneumoniae (5e−37). Of the biogenesis genes, pilBCD, pilB, which encodes the ATPase, is the best conserved (E values = 0.0), and has well conserved homologues outside the family Pasteurellaceae (e.g., in Plesiomonas shigelloides, E value = 5e−20). On the other hand, the pilD gene, predicted to encode the fimbrial leader peptidase, is not conserved in species outside the family Pasteurellaceae (e.g., Enterococcus faecium, E value = 0.014).

Like pilABCD/apfABCD, the comABCDEF competence locus is predicted to encode the biogenesis components for type IV pilus assembly; however, no pilin gene is associated with this operon in the A. suis genome, and the comF gene (ASU2_11115) is not linked with the rest of the com locus, unlike other species such as NTHi [24]. In a recent study of NTHi, Carruthers et al. found that all of the products of both the pil and com operons, including comF, are essential for proper type IV pilus construction and formation [24]. Taken together, these results suggest that the proteins encoded by the pil and com loci may work together to produce type IV pili in A. suis, and that the pilA homologue (ASU2_05045) may encode the major pilin protein.
Outer membrane proteins

Genes predicted to encode twelve outer membrane proteins (OMPs) were identified, including homologues ofompA, ompP2, andompP5 porin genes (Table 3). OMPs are described as multifunctional proteins. Many OMPs have been demonstrated to form porins in the outer membrane of Gram negative bacteria, which can contribute to nutrient acquisition, antibiotic resistance,
| ASU2 locus tag | GenInfo (GI) number | (Possible) gene name | Annotated protein function | Top App homologue/ E value | Top Pasteurellaceae homologue/E value | Top other homologue/E value |
|---------------|---------------------|----------------------|-----------------------------|----------------------------|-------------------------------------|-----------------------------|
| ASU2_06635    | 407388972            | (fhaB)<sup>c</sup>   | Filamentous haemagglutinin outer membrane protein | Ser. 6 str. Femo 0.0       | Actinobacillus capsulatus 0.0       | Acinetobacter bohemicus 0.0   |
| ASU2_06640    | 407388973            | (fhoC)<sup>c</sup>   | Hemolysin activation/secretion protein | Ser. 4 str. M62 0.0        | Actinobacillus capsulatus 0.0       | Ralstonia solanacearum 1e−168 |
| ASU2_09130    | 407389463            | (ftpA, dpA)<sup>c</sup> | Fine tangled pili major subunit; DNA-binding ferritin-like protein; DNA protection during starvation | Ser. 3 str. JL03 2e−119    | Actinobacillus ureae 2E−130      | Jonesia denitrificans DSM 20603 |
| ASU2_10345    | 407389704            | (comE1, comEA, ybaV)<sup>c</sup> | DNA uptake protein; DNA uptake protein and related DNA-binding proteins; transporter | Ser. 7 str. AP76 6e−34     | Actinobacillus capsulatus 1E−75   | Vibrio nigripulchritudo 4e−21  |

<sup>a</sup> indicates a suggested name that was not present in the annotation  
<sup>b</sup> Function assigned by conserved motifs  
<sup>c</sup> Identified by BASys  
<sup>d</sup> Classified by description of homologues
attachment, invasion, and complement resistance, to name a few [35].

Most of the OMPs of *A. suis* are highly conserved when compared to other members of the family *Pasteurellaceae*. Two members of the OmpA family were identified in the *A. suis* genome, ASU2_09940 and ASU2_09935. In our previous studies, the OmpA homologue ASU2_09940 was identified by signature-tagged mutagenesis as an important virulence factor of *A. suis*, with a demonstrated role in attachment to swine tonsil explants and to porcine brain microvascular endothelial cells [36, 37]. The other member of the OmpA family of OMPs, an *ompP5* homologue (ASU2_09935), is adjacent to the *ompA* homologue in the *A. suis* genome. It is also highly conserved (E value = 0.0) in members of the family *Pasteurellaceae* and has a high degree of homology with OMPs outside the family. In *NTHi*, *OmpP5* has been shown to bind to human mucin [38] and to CEACAM1 [39]; however, the precise role of OmpP5 and most other *A. suis* OMPs in pathogenesis remains to be demonstrated.

Two *ompP2* genes (ASU2_00030 and ASU2_00525) and one *ompP2*-like gene (ASU2_003810) were identified in the *A. suis* genome. In addition to conferring antibiotic resistance [40], providing a pore for general diffusion and transport of specific substrates [41], the OmpP2 of *NTHi* has also been shown to play a role in attachment in the host environment through interactions with mucin [42]. The *ompP2* gene (ASU2_00030) is predicted to encode a protein that is very similar to an *ompP2* homologue in *A. capsulatus* (E = 0.0) while the ASU2_00525 gene encodes a protein that is well conserved in *A. ureae* (9E−159). The *ompP2* homologues identified in *A. suis* are well conserved in *A. pleuropneumoniae*, but the serovar of the top homologues in *A. pleuropneumoniae* is different with each gene, as is the degree of conservation. Of the OMPs identified in *A. suis*, the *ompP2* gene ASU2_00525 and the *plp4* homologue ASU2_02415 have the least homology with proteins encoded by organisms outside of the family *Pasteurellaceae*. The GC content of ASU2_00525 differs markedly from that of the *A. suis* genome (36 vs. 40.24 %), which may suggest that this *ompP2* gene was recently acquired by *A. suis*.

Because of the multifunctional nature of the OMPs, it would be premature to predict that all OMPs identified in this study play a role in attachment or invasion, and further studies should be done to characterise each gene and its potential role in bacterial pathogenesis for *A. suis*.

Miscellaneous adhesins

Four additional genes from three different loci were identified that could play a role in bacterial attachment, colonisation, or invasion for *A. suis* (Table 4).

A filamentous haemagglutinin (FHA) locus consisting of two genes (ASU2_06635 and ASU2_06640) is also found in the *A. suis* genome. The *fhaB* gene encodes the adhesin structure while *fhaC* encodes the transporter. FHA has been demonstrated to play a role in bacterial attachment to integrins, carbohydrates present on macrophages, cilia, epithelial cells, and extracellular matrix components including heparin [43], and is thought to contribute to colonisation and biofilm formation by important pathogens such as *Histophilus somni*, *Bordetella bronchiseptica*, *Acinetobacter baumannii*, and *Pasteurella multocida* [44–47]. The *A. suis fhaB* gene has highly conserved (E values = 0.0) homologues in *A. pleuropneumoniae*, *Pasteurellaceae*, and in other species outside of the family *Pasteurellaceae*. The *fhaC* gene is also predicted to encode highly conserved homologues in members of the family *Pasteurellaceae* but to a slightly lower degree (E value = 1e−168). It is also interesting to note that the TAA-encoding ASU2_06645 gene is linked to the filamentous haemagglutinin locus, though the relevance of this finding, if any, remains to be elucidated.

A fine-tangled pilus gene, *ftpA*, is also present in the *A. suis* genome. This gene lacks a cleavable signal sequence [48], and no biogenesis genes for the translocation and assembly of this structure were identified. In other species, fine-tangled pili are assigned to the DNA protection during starvation (DPS) family of proteins. DPS proteins are thought to confer protection of DNA from environmental stressors such as low pH, Fe^{3+}, and hydrogen peroxide [49]. Further, these proteins have been shown to be involved in bacterial adhesion to and invasion of host cells, and in auto-aggregation [49–53], though it is not clear whether the mechanisms of these actions are via a direct or indirect adhesive function of the Dps homologue. In *A. suis*, the *ftpA* gene (ASU2_09130) is well conserved in both *A. pleuropneumoniae* (E value = 2e−119) and other members of Pasteurellaceae (*A. ureae*, E value = 2e−130), and to a lesser extent in other species (*Jonesia denitrificans*, E value = 2e−61).

Finally, a homologue of *comE1*, originally described in *Pasteurella multocida* [54], was also identified in *A. suis*. In addition to its roles in DNA-binding and uptake, this gene encodes a protein involved in bacterial attachment of five different members of the family *Pasteurellaceae* to the extracellular matrix component fibronectin [55, 56]. The closest homologue of the *comE1* gene in *A. suis* (ASU2_10345) is found in *A. capsulatus* (E value = 1e−75). Less well conserved homologues are also present in *A. pleuropneumoniae* (E value = 6e−34), other members of *Pasteurellaceae*, and even in other species outside the family. Given the role of this gene in fibronectin-binding in other members of *Pasteurellaceae*,
it would be interesting to assess whether it plays a similar function in *A. suis*.

**Adhesins in other *A. suis* strains**

To determine whether putative adhesin genes are conserved in other *A. suis* isolates, real-time PCR was done on 9 additional isolates, including *A. pleuropneumoniae* L20, a serovar 5b isolate (Table 5). Ten genes were chosen for characterisation, with representatives from each of the classes of adhesins described. All *A. suis* isolates tested were positive for the selected adhesin genes, while the *A. pleuropneumoniae* isolate was only positive for the putative *ompP2* gene. Upon closer inspection of the *A. pleuropneumoniae* L20 genome sequence, the only adhesin gene tested without a homologue was *ycgV* (ASU2_07665); however, despite there being homologues of the other genes, the sequence conservation in the primer binding sites in all but the *ompP2* gene was poor.

The pseudogenomes of three additional *A. suis* genomes—ATCC 15557, H89-0406, and H91-1173—were annotated using BASys, and the genome sequence of ATCC 33416 was obtained from GenBank [57]. These four genome sequences were used to determine whether putative adhesin genes were conserved in different *A. suis* isolates using blastn for direct nucleotide sequence comparisons (Additional file 1). Homologues of all adhesin genes identified in the *A. suis* H91-0380 genome were found in the four additional genomes, and were for the most part highly conserved (most >99% sequence identity). Some gene lengths varied among isolates, with the most notable differences seen in the ASU2_04675 autotransporter-encoding homologue found in ATCC 33415 and ATCC 15557, the ASU2_11275 autotransporter-encoding homologue in ATCC 15557, and the truncated but highly conserved *flp1* (ASU2_04295) homologue in H89-1173. The OMP homologue ASU2_01965 in the ATCC 33415 isolate shared only 67% nucleotide identity with H91-0380, despite 90% sequence coverage. Overall, however, putative adhesin genes were highly conserved in all *A. suis* isolates examined, which may suggest a clonal population, though other classes of genes, particularly virulence-associated genes, should also be compared.

**Conclusions**

Attachment and colonisation of the host environment are important steps in the early stages of bacterial colonisation and pathogenesis [7]. As virtually nothing was known about these early steps in *A. suis*, the purpose of this study was to identify putative adhesins that may contribute to these processes in the genomes of several *A. suis* strains. Our analysis revealed that *A. suis* shares many of the same putative adhesins as *A. pleuropneumoniae*, an important primary pathogen of swine that is also known to colonise the upper respiratory tract. It may therefore be hypothesised that the different tissue tropisms and diseases caused by *A. suis* and *A. pleuropneumoniae* might be attributed, at least in part, to subtle differences in the adhesins of these organisms or to differential expression of adhesins at different stages of the infection process.

The adhesins identified in the *A. suis* genome are also well conserved in several other members of the family Pasteurellaceae. It is perhaps noteworthy that *Pasteurellaceae* that cause similar diseases but in different hosts, such as *A. ureae* and *A. capsulatus*, have nearly all the same adhesins as are present in *A. suis*. Of particular note are the autotransporter-encoding genes *ycgV* and *aidA* that are present in *A. suis*, *A. ureae*, and *A. capsulatus*, but which are missing in *A. pleuropneumoniae*. It may be hypothesised that these organisms employ similar strategies to invade the host, but more work is needed to characterise such host-pathogen interactions.

Together, these data begin to identify attachment and colonisation factors that may allow some members of the family Pasteurellaceae to invade the bloodstream and others to cause more localised infections. Future research on the expression of adhesins in *A. suis* and other organisms will help in elucidating the mechanisms of attachment and colonisation, and should eventually lead to a better understanding of critical host-pathogen relationships.

**Methods**

**Bioinformatics**

To identify putative adhesin-associated genes in *Actinobacillus suis* H91-0380, a virulent O2:K2 isolate [6], a manual search of the annotations of the *A. suis* H91-0380 genome assigned by the BASys pipeline [58] and GenBank (http://www.ncbi.nlm.nih.gov/) was done to identify putative adhesin-associated genes; blastx was used to find homologues in other species with a described or annotated role in attachment, colonisation, or invasion. Genes or proteins described in the literature in other members of the family Pasteurellaceae were also analysed by blastx or blastp to find homologues in *A. suis*.

Further analysis of selected putative adhesin-associated genes was done using Pfam (http://pfam.xfam.org/) to determine if conserved amino acid motifs characteristic of described protein families were present. When motifs were not identified, sequence identity and query coverage alone were used to classify genes.

**Bacterial strains and growth media**

Bacterial isolates (Table 6) were cultured from glycerol stocks onto Columbia agar plates containing 5%
Table 5  Real-time PCR detection of selected putative adhesin genes with *A. suis* H91-0380 primers

| Isolate   | YcgV (ASU2_07665) | Flp1 (ASU2_04295) | TadG (ASU2_04360) | PilA (ASU2_05045) | OmpP2 (ASU2_00030) | OmpA (ASU2_09940) | Plp4 (ASU2_11270) | FhaB (ASU2_06635) | FtpA (ASU2_09130) | ComE1 (ASU2_10345) |
|-----------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|------------------|------------------|------------------|
| H91-0380  | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| ATCC 15557| +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| H91-0406  | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| SO4 NaI   | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| VSB 3714  | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| CB4       | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| Q95-6256  | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| H93-1250  | +                 | +                 | +                 | +                 | +                 | +                | +                 | +                | +                | +                |
| App L20   | −                 | −                 | −                 | −                 | −                 | −                | −                 | −                | −                | −                |

+, indicates detection of a gene in a specific isolate

−, indicates no detection of a gene in a specific isolate
sheep's blood (Oxoid Co., Nepean, ON, USA), and in the case of the *A. pleuropneumoniae* isolate, supplemented with 0.01 % (wt/vol) nicotinamide adenine dinucleotide (Sigma-Aldrich, St. Louis, MO). Plates were incubated overnight at 37 °C in an atmosphere of 5 % CO₂.

### Real-time PCR

Crude genomic DNA was prepared by picking isolated colonies and dispersing them in Instagene matrix (Bio-Rad Laboratories Ltd., Hercules, CA), mixing by vortex, incubating at 56 °C for 30 min, mixing again by vortex, incubating at 100 °C for 8 min, centrifuging at 5000 × g for 2 min, and using the supernatant as template for PCR. At least two biological replicates were done for each strain and gene tested.

PCR primers were designed using Primer3 as previously described [59], and are listed in Table 7. The total reaction volume was 20 µL, which contained 10 µL Light-Cycler 480 SYBR Green I Master mix (Roche Diagnostics Co., Indianapolis, IN, USA), 0.4 µL each of the forward and reverse primers to a final concentration of 1 µM, 4.2 µL nuclease-free water, and 5 µL template.

### Table 6 Strains used in this study

| Bacterial strain                | Characteristic(s) | GenBank accession number and Reference |
|---------------------------------|-------------------|----------------------------------------|
| *Actinobacillus suis* H91-0380  | O2:K2 clinical isolate | CP003875; [6, 61]                     |
| *Actinobacillus suis* ATCC 33415| Untyped clinical isolate | CP009159; [57]                      |
| *Actinobacillus suis* ATCC 15557| O1:K1 isolate       | [61, 62]                               |
| *Actinobacillus suis* H89-1173  | O2:K3 clinical isolate | [61, 62]                               |
| *Actinobacillus suis* H91-0406  | O2:K2 clinical isolate | [61, 62]                               |
| *Actinobacillus suis* SO4 Naïf | O1:K1 isolate       | [61, 62]                               |
| *Actinobacillus suis* VSB 3714  | Rough K7 isolate   | [61, 62]                               |
| *Actinobacillus suis* C84      | O1:K2 isolate       | [61, 62]                               |
| *Actinobacillus suis* Q95-6256 | Untypable isolate  | [61, 62]                               |
| *Actinobacillus suis* H93-1250 | Untyped clinical isolate | [61, 62]                               |
| *Actinobacillus pleuropneumoniae* L20 | Serovar 5b      | [63]                                   |

### Table 7 Primers used in this work

| Primer name   | Class               | Locus tag   | Sequence                  | Source       |
|---------------|---------------------|-------------|---------------------------|--------------|
| ASU2-ycgV-F1  | Autotransporter     | ASU2_07665  | CTTGGGATGTGTCCGTGTTGCT    | This work    |
| ASU2-ycgV-R1  |                     |             | TTTACCCGGAGTTATCGTACCGT   | This work    |
| ASU2-flp1-F1  | Fimbriae-associated | ASU2_04295  | CTGTAACCTGAAGATCGCAACT    | This work    |
| ASU2-flp1-R1  |                     |             | TGCCTGAACCAAGCTAAACTACG   | This work    |
| ASU2-tadG-F1  | Fimbriae-associated | ASU2_04360  | GCAGAGTACTGAAGCAGACTC     | This work    |
| ASU2-tadG-R1  |                     |             | ACTGATTACAGGACACATTG      | This work    |
| ASU2-pilA-F1  | Fimbriae-associated | ASU2_05045  | ACTGTAAGGGGCATCTTGCT      | This work    |
| ASU2-pilA-R1  |                     |             | CTACCCTGCTGCTTACGATC      | This work    |
| ASU2-ompP2-F1 | OMP                 | ASU2_0030   | ACCCTAGGGCAAGACACTTTAACA  | This work    |
| ASU2-ompP2-R1 |                     |             | TAAACGGGCTTCTACCGGCTTAAC  | This work    |
| ASU2-ompA-F1  | OMP                 | ASU2_09940  | CGGTAAGATGGTGTTGACGTT     | This work    |
| ASU2-ompA-R1  |                     |             | ATTTCTCTTGTTGCTGTTAAG     | This work    |
| ASU2-pilP4-F1 | OMP                 | ASU2_11270  | GTCGAATCTACTGGAAGGTTAAG   | This work    |
| ASU2-pilP4-R1 |                     |             | GTTGATGAGGAGACATACCGG     | This work    |
| ASU2-fhaB-F1  | Miscellaneous       | ASU2_06635  | GGGATTTAGGGTGTTGAGT       | This work    |
| ASU2-fhaB-R1  |                     |             | ATACTTTGTCTTGGTTGAGGCT    | This work    |
| ASU2-rfaA-F1  | Miscellaneous       | ASU2_09130  | CGGACGCTGATTGAGCATGATT    | This work    |
| ASU2-rfaA-R1  |                     |             | GGTATTTACGCGTGGTGAGT      | This work    |
| ASU2-comE1-F1 | Miscellaneous       | ASU2_10345  | GTCAACGAAAACCACCTCCGGT    | This work    |
| ASU2-comE1-R1 |                     |             | TTTATCTGATTTCCGCTGT      | This work    |
Real-time PCR was done in a LightCycler 480 (Roche Diagnostics Co., Indianapolis, IN) using a program with an initial denaturation of 95 °C for 5 min followed by 45 cycles of 95 °C for 10 s, 54 °C for 20 s, and 72 °C for 12 s. Stepwise melt curves were done at the end of each run to confirm that only one template was amplified.

### Sequencing additional isolates

*Actinobacillus suis* strains ATCC 15557, H89-1173, and H91-0406 were sequenced at the Advanced Analytics Centre at the University of Guelph using MiSeq, and pseudogenomes were assembled with SeqMan Pro (DNASTAR Inc., Madison, WI, USA) followed by progressiveMauve [60], and annotated using the BASys pipeline [58].

### Additional file

**Additional file 1.** blastn comparison of *A. suis* H91-0380 adhesin-associated genes to four additional *A. suis* strains. Spreadsheet of blast results showing gene sizes, locations, query coverage, E value, and sequence identity for adhesin-associated genes in *A. suis* H91-0380 compared to *A. suis* ATCC 33415, H91-0406, ATCC 15557, and H89-1173.

### Authors' contributions

ARB designed and carried out data collection and analysis, and drafted the manuscript. JIM assisted in experimental design. Both authors read and approved the final manuscript.

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### Competing interests

The authors declare that they have no competing interests.

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