In Silico Detection of Virulence Gene Homologues in the Human Pathogen *Sphingomonas* spp.

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ABSTRACT: There is an ongoing debate about the clinical significance of *Sphingomonas paucimobilis* as a virulent bacterial pathogen. In the present study, we investigated the presence of different virulence factors and genes in *Sphingomonas* bacteria. We utilized phylogenetic, comparative genomics and bioinformatics analysis to investigate the potentiality of *Sphingomonas* bacteria as virulent pathogenic bacteria. The 16S ribosomal RNA gene (16S rDNA) phylogenetic tree showed that the closest bacterial taxon to *Sphingomonas* is *Brucella* with a bootstrap value of 87 followed by *Helicobacter, Campylobacter, Pseudomonas*, and then *Legionella*. *Sphingomonas* shared no virulence factors with *Helicobacter* or *Campylobacter*, despite their close phylogenetic relationship. In spite of the phylogenetic divergence between *Sphingomonas* and *Pseudomonas*, they shared many major virulence factors, such as adherence, antiphagocytosis, iron uptake, proteases, and quorum sensing. In conclusion, *Sphingomonas* spp. contains several major virulence factors resembling *Pseudomonas* sp., *Legionella* sp., *Brucella* sp., and *Bordetella* sp. virulence factors. Similarity of virulence factors did not match phylogenetic relationships. These findings suggest horizontal gene transfer of virulence factors rather than sharing a common pathogenic ancestor. *Sphingomonas* spp. is potential virulent bacterial pathogen.

KEYWORDS: *Sphingomonas* spp., virulence factors, phylogenetics, comparative genomics, bioinformatics, *Pseudomonas* sp.

Introduction

*Sphingomonas* is a group of Gram-negative, rod-shaped, non-spore-forming, chemoheterotrophic, strictly aerobic bacterium that produces yellow or off-white pigmented colonies. The distinctiveness of *Sphingomonas* lies in its possession of ubiquinone 10 as its major respiratory quinone, presence of glycosphingolipids (GDLs) in their cell envelopes, and its metabolic versatility.¹ The genome of *Sphingomonas* is approximately 3,948 kB and contains 70 structural RNAs. It encodes for approximately 3,914 proteins.² *Sphingomonas* utilize glucose as its primary source of carbon. However, it can also utilize a wide variety of other sugars such as arabinose, fucose, glucose, lactose, mannose, melibiose, sucrose, trehalose, and xylene. Moreover, *Sphingomonas* can also degrade polysaccharides, and many of the strains were also able to utilize one or more of the contaminants as their source of carbon.² The capability of *Sphingomonas* to utilize a wide range of organic compounds, and to grow and survive under low-nutrient conditions has resulted in its widespread distribution in various environments, including drinking water, soil, air, sinks, shower curtains, and corroding copper pipes. In addition, *Sphingomonas paucimobilis* has also been found in several clinical specimens, which include hospital water supplies; temperature probe respirators; stocked distilled water; blood; removes; hospital dialysis equipment; patients with meningitis, septicemia, bacteremia, and peritonitis; and wound infections.³ Reported cases of nosocomial infections caused by *S. paucimobilis* are rarely serious and could be effectively...
treated with antibiotics. On the contrary, some other reports have concluded that *S. paucimobilis* nosocomial infections have the ability to severely threaten immune-compromised or ill patients causing health problems with consistent exposure to the source of infection such as shower curtains. For example, in 2007, reports concluded that *S. paucimobilis* was the cause of bacteremia outbreak in the hemato/oncology units in Gülhane Military Hospital in Ankara, Turkey. The reports also showed that the clinical isolates were traced back neither to the health care workers nor to the environmental isolates. Moreover, it was strongly documented that *S. paucimobilis* created significant problems in various clinical settings, being the most widespread cause of nosocomial infections including bacteraemia/septicemia caused by contaminated solutions such as distilled water, hemodialysis fluid, and sterile drug solutions. Cases of pseudo-bacteremia have been recorded in association with *S. paucimobilis*, as have many cases of unusual infections both invasive and severe, eg, septic arthritis and osteomyelitis. Moreover, *S. paucimobilis* caused bloodstream infection in a patient with Down syndrome. It was thereby concluded that *S. paucimobilis* should be recognized as a nosocomial infectious agent in patients with Down syndrome and immunosuppressive disorders. In addition, it was also reported that *S. paucimobilis* has the ability to cause infections in both previously healthy and immune-compromised children and can act as a causal agent of osteomyelitis in an immune-competent patient. Frequent *S. paucimobilis* infections were observed among our diabetic foot ulcer patients (23% of observed Gram-negative infections). *S. paucimobilis* general infection rate was 9.5%, falling just behind *Staphylococcus aureus* (unpublished data). There is still an ongoing debate about the clinical virulence of *S. paucimobilis* with a possible conclusion that its clinical importance cannot be neglected. Henceforth, this study employs comparative genomics and bioinformatics techniques in order to investigate the pathogenic potentials of *Sphingomonas* spp.

Materials and Methods

**Phylogenetic relationships reconstruction.** Partial 16S rDNA sequences of selected pathogenic bacteria, namely, *S. paucimobilis* (D16144.1), *Bacillus anthracis* (GQ280034.1), *Bartonella bacilliformis* (AF442955.1), *Brucella* sp. (DQ413258.1), *Burkholderia* sp. (AB379686.1), *Campylobacter jejuni* (AY621112.1), *Clostridium difficile* (HM245939.1), *Corynebacterium* sp. (D33735.1), *Escherichia coli* (KC504012.1), *Haemophilus* sp. (AB004027.1), *Helicobacter* sp. (AY034821.1), *Legionella pneumophila* (NR_041742.1), *Neisseria meningitidis* (AF059671.1), *Pseudomonas* sp. (AB379690.1), *Salmonella typhimurium* (DQ153191.1), *Shigella* sp. (JN626189.1), *Vibrio cholerae* (Z21856.1), *Yersinia pestis* (AJ232235.1), were all acquired from the GenBank. These sequences were then aligned using the Bioedit built-in clustal W program (gap opening penalty = 10, gap extension penalty = 5, delay divergent sequences = 40%). The resulting alignments were compared, and the final alignments were improved manually and prepared in FASTA and MEGA formats using format converter tool v2.2.5 available online at http://www.hiv.lanl.gov/content/sequence/FORMAT_CONVERSION/form.html.

In order to establish the phylogenetic relationships among taxa, tree was constructed using the maximum likelihood (ML) method based on the Tamura–Nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search was(were) obtained automatically by applying Neighbor-Joining and BIONJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then the topology was selected with superior log-likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences. Codon positions included were first + second + third + noncoding. All positions containing gaps and missing data were eliminated. There were a total of 253 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

**Comparative genomics and bioinformatics analysis.** Virulence genes sequences and functions, corresponding to different major bacterial virulence factors of selected pathogens, were collected from GenBank and validated in virulence factors of pathogenic bacteria database at http://www.mgc.ac.cn/VFs/. Supplementary Table 1 shows the tested major pathogenic virulence factors. Selected gene sequences were tested against available *Sphingomonas* gene information using *Sphingomonas* nucleotide BLAST tool available at http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&BLAST_SPEC=MicrobialGenomes_13687&DB_GROUP=AllM. The search set all *Sphingomonas* complete genomes, and selected organism was *Sphingomonas* (tax Id: 13687). The program selected for the search was blastn, optimizes for fairly similar sequences because of evolutionary divergence of the tested and query taxa.

Results and Discussion

In this study, the presence of the major known bacterial virulence factors in *Sphingomonas* spp. was examined. In order to decide on the accurate common pathogenic bacterial species for comparison with *Sphingomonas* spp., a phylogenetic tree using the ML method was constructed using partial 16S rDNA sequences of selected pathogenic bacteria as mentioned above (Fig. 1). The phylogenetic tree showed that the selected bacterial species are divided into two major clades (groups), namely, Gram-positive bacteria and Gram-negative bacteria. The Gram-positive bacterial group contained *Clostridium* spp., *Corynebacterium* sp., and *Bacillus* sp., whereas the Gram-negative bacterial group contained the remaining bacterial species. These results also agree with the known taxonomic arrangement of the tested bacterial species with the
Comparative genomics analysis of *sphingomonas* virulence factors

The comparison of the 16S rDNA phylogenetic tree showed *Brucella* sp. to be the closest bacterial taxon to *Sphingomonas* with a bootstrap value of 87 followed by *Helicobacter* spp., *Campylobacter* sp., *Pseudomonas* sp., and then *Legionella* sp. Based on the suggested phylogenetic relationships, the following bacterial species which include *Brucella* sp., *Helicobacter* spp., *Campylobacter* sp., *Pseudomonas* sp., and *Legionella* sp. were selected for further comparative genomic and bioinformatics analyses.

| GENUS      | SPECIES         | HOST                   | DISEASE                                      |
|------------|-----------------|------------------------|----------------------------------------------|
| *Brucella* | *B. abortus*    | Human and cattle       | Brucellosis, Osteoarthritis, and endocarditis |
|            | *B. canis*      | Human and dogs         |                                             |
|            | *B. melitensis* | Human goats and sheep  |                                             |
|            | *B. ovis*       | Sheep                  |                                             |
|            | *B. suis*       | Human and pigs         |                                             |
| *Helicobacter* | *H. acinonychis* | Humans and other mammals | Bacterial carcinogen, Gastroduodenal diseases |
|            | *H. hepaticus*  | Humans                 |                                             |
|            | *H. pylori*     | Humans                 |                                             |
| *Campylobacter* | *C. fetus*    | Humans                 | Bacterial gastroenteritis                  |
|            | *C. jejuni*     | Humans                 | Guilain-Barre syndrome (GBS)                |
| *Legionella* | *L. pneumophila* | Humans and protozoa   | Legionnaires’ disease                       |
| *Pseudomonas* | *P. aeruginosa* | Human                  | Eye, burn and wound infections              |
|            | *P. syringae*   | Plant                  | Bacterial speck and bacterial blight       |

Regardless of the phylogenetic divergence between *Sphingomonas* spp. and *Pseudomonas* sp., it was observed from our results that they shared several major virulence factors such as adherence, endotoxin, mobility, secretion systems, quorum sensing, and many others are shown in (Table 2). Table 3 presents the five selected bacterial genera with its corresponding species, the selected hosts, and the diseases caused by these pathogens. The selected pathogens were mainly human pathogens having also the ability to infect mammals, protozoa (*Legionella* sp.), and plants (*Pseudomonas syringae*). All the virulent factors acquired by these pathogens (Table 2) were tested for their presence in *Sphingomonas* genomic information. The major categories of bacterial virulence factors such as adherence, endotoxin, mobility, secretion systems, quorum sensing, and many others are shown in (Table 2).

The 16S rDNA phylogenetic tree showed *Brucella* sp. to be the closest bacterial taxon to *Sphingomonas* with a bootstrap value of 87 followed by *Helicobacter* spp., *Campylobacter* sp., *Pseudomonas* sp., and then *Legionella* sp. Based on the suggested phylogenetic relationships, the following bacterial species which include *Brucella* sp., *Helicobacter* spp., *Campylobacter* sp., *Pseudomonas* sp., and *Legionella* sp. were selected for further comparative genomic and bioinformatics analyses. Table 1 presents the five selected bacterial genera with its corresponding species, the selected hosts, and the diseases caused by these pathogens. The selected pathogens were mainly human pathogens having also the ability to infect mammals, protozoa (*Legionella* sp.), and plants (*Pseudomonas syringae*). All the virulent factors acquired by these pathogens (Table 2) were tested for their presence in *Sphingomonas* genomic information. The major categories of bacterial virulence factors such as adherence, endotoxin, mobility, secretion systems, quorum sensing, and many others are shown in (Table 2).
Table 2. Major pathogenic virulence factors and its corresponding genes and functions used in the comparative analysis against *Sphingomonas* spp.

| GENUS        | MAJOR TESTED VIRULENCE FACTORS | CORRESPONDING TESTED GENES OR FUNCTION |
|--------------|--------------------------------|---------------------------------------|
| *Brucella*   | Immune-evasion                 | Btp1/TcpB (Toll-interleukin-1 receptor (TIR) domain) |
|              | Intracellular survival          | Cyclic β-[1,2-glucan synthase (Cgs), gmd; manA; manC; per; pgm; pmm/manB; wbkA; wbkB; wbkC; wzm; wzl; and RiaC (Rab2 interacting conserved protein A)) |
|              | Regulation                      | BvrR-BvrS two-component system         |
|              | Secretion system                | BMEII0025; BMEII0026; BMEII0027; BMEII0028; BMEII0029; BMEII0030; BMEII0031; BMEII0032; BMEII0033; BMEII0034; BMEII0035; type IV secretion system |
| *Helicobacter* | Adherence                      | babA; babB; hopZ; sabA; |
|              | Endotoxin                      | gluE; gluP; kdtB; lpxB; rfaC; rfaJ; rfdB; rfbM; wbcJ; wbpB; |
|              | Enzyme                          | ureA; ureB; ureE; ureF; ureG; ureH; ureI; |
|              | Molecular mimicry               | fucT; fucU; neuA; neuB; |
|              | Motility                        | flaA; flaB; flgG |
|              | Proinflammatory effect          | napA; oipA; |
|              | Secretion system                | cag1; cag10; cag11; cag12; cag13; cag14; cag15; cag16; cag17; cag18; cagL; cag19; cag2; cag20; cag21; cag22; cag23 (cagE/cagB); cag24; cag25; cag3; cag4; cag5 (virD4); cag6; cag7; cag8; cag9; virB11; type IV secretion system. |
|              | Toxin                           | vacA |
|              | Type IV secretory protein       | cagA |
|              | Pathogenicity islands           | cag-PAI |
| *Campylobacter* | Adherence                      | cadF; Cj1415c; Cj1416c; Cj1421c; Cj1422c; Cj1433c; Cj1434c; Cj1435c; Cj1436c; Cj1437c; Cj1438c; Cj1440c; Cj1442c; cfl; gfh; gfhA; gfhB; gfhC; gfhD; gfhE; gfhF; gfhG; gfhH; gfhI; gfhJ; gfhK; gfhL; gfhM; gfhN; gfhO; gfhP; gfhQ; gfhR; gfhS; gfhT; gfhU; gfhV; gfhW; gfhX; gfhY; gfhZ; glf; gmha; htrB; neuA1; neuB1; neuC1; waaC; waaD; waaE; waaF; waaV; porA; pbe1A. |
|              | Invasion                        | ciaB, ciaC (invasion antigens) |
|              | Motility                        | Cj0371; Cj1312; Cj1313; flaA; flaB; flaC; flaD; flaG; flaB; flaC; flaD; flaE; flaF; flaG; flaH; flaI; flaJ; flaK; flaL; flaM; flaN; flaO; flaP; flaQ; flaR; flaS; flaT; flaU; flaV; flaW; flaX; flaY; flaZ; flhA; flhB; flhC; flhD; flhE; flhF; flhG; flhH; flhI; flhJ; flhK; flhL; flhM; flhN; flhO; flhP; flhQ; flhR; flhS; flhT; flhU; flhV; flhW; flhX; flhY; flhZ; flgA; flgB; flgC; flgD; flgE; flgF; flgG; flgH; flgI; flgJ; flgK; flgL; flgM; flgN; flgO; flgP; flgQ; flgR; flgS; flgT; flgU; flgV; flgW; flgX; flgY; flgZ; fliA; fliB; fliC; fliD; fliE; fliF; fliG; fliH; fliJ; fliK; fliL; fliM; fliN; fliO; fliP; fliQ; fliR; fliS; fliT; fliU; fliV; fliW; fliX; fliY; fliZ; fimT; fimU; fimV; pilA; pilB; pilC; pilD; pilE; pilF; pilG; pilH; pilI; pilJ; pilK; pilL; pilM; pilN; pilO; pilP; pilQ; pilR; pilS; pilT; pilU; pilV; pilW; pilX; pilY1; pilY2; |
|              | Secretion system                | Cj54; virB10; virB11; virB13; virB4; virB8; virB9; virD4; type IV secretion system. |
|              | Toxin                           | cdtA; cdtB; cdtC; |
| *Legionella* | Adherence                       | htpB; omp28; pilB; pilC; pilD; |
|              | Enzyme                          | mip; |
|              | Iron uptake                      | ccmC; icaAB; fragA; foa; foEO; |
|              | Motility                        | flaA; flaB; flaC; flaD; flaE; flaF; flaG; flaH; flaI; flaJ; flaK; flaL; flaM; flaN; flaO; flaP; flaQ; flaR; flaS; flaT; flaU; flaV; flaW; flaX; flaY; flaZ; flhA; flhB; flhC; flhD; flhE; flhF; flhG; flhH; flhI; flhJ; flhK; flhL; flhM; flhN; flhO; flhP; flhQ; flhR; flhS; flhT; flhU; flhV; flhW; flhX; flhY; flhZ; flgA; flgB; flgC; flgD; flgE; flgF; flgG; flgH; flgI; flgJ; flgK; flgL; flgM; flgN; flgO; flgP; flgQ; flgR; flgS; flgT; flgU; flgV; flgW; flgX; flgY; flgZ; fliA; fliB; fliC; fliD; fliE; fliF; fliG; fliH; fliJ; fliK; fliL; fliM; fliN; fliO; fliP; fliQ; fliR; fliS; motA; motB; |
|              | Nutrient acquisition             | phtA; |
|              | Regulation                      | csrA; letA; letS; relA; pso; |
|              | Secretion system                | islD; islP; islS; islT; islV; islW; islX; islY; islZ; pilA; (Type II secretion system). dotA; dotB; dotC; dotD; dotE; dotF; dotG; dotH; dotI; dotJ; dotK; dotL; dotM; dotN; dotO; dotP; dotQ; dotR; dotS; dotT; dotU; dotV; dotW; dotX; dotY; dotZ; fimT; fimU; fimV; pilA; pilB; pilC; pilD; pilE; pilF; pilG; pilH; pilI; pilJ; pilK; pilL; pilM; pilN; pilO; pilP; pilQ; pilR; pilS; pilT; pilU; pilV; pilW; pilX; pilY1; pilY2; |
|              | Stress protein                  | gspA (global stress gene) katA; katB; sodB; sodC; |
|              | Toxin                           | RtxA; |
|              | Unclassified                    | enhA; enhB; enhC; ligA; |
| *Pseudomonas* | Adherence                       | fleA; fleB; fleC; fleD; fleE; fleF; fleG; fleH; fleI; fleJ; fleK; fleL; fleM; fleN; fleO; fleP; fleQ; fleR; fleS; fleT; fleU; fleV; fleW; fleX; fleY; fleZ; flhA; flhB; flhC; flhD; flhE; flhF; flhG; flhH; flhI; flhJ; flhK; flhL; flhM; flhN; flhO; flhP; flhQ; flhR; flhS; flhT; flhU; flhV; flhW; flhX; flhY; flhZ; flgA; flgB; flgC; flgD; flgE; flgF; flgG; flgH; flgI; flgJ; flgK; flgL; flgM; flgN; flgO; flgP; flgQ; flgR; flgS; flgT; flgU; flgV; flgW; flgX; flgY; flgZ; fliA; fliB; fliC; fliD; fliE; fliF; fliG; fliH; fliI; fliJ; fliK; fliL; fliM; fliN; fliO; fliP; fliQ; fliR; fliS; motA; motB; |

(Continued)
### Table 2 (Continued)

| GENUS | MAJOR TESTED VIRULENCE FACTORS | CORRESPONDING TESTED GENES OR FUNCTION |
|-------|----------------------------------|---------------------------------------|
|       | Antiphagocytosis                  | Alg44; Alg8; algA; algB; algD; algE; algF; algG; algI; algJ; algK; algL; algP; algQ; algR; algU; algX; algZ; mucA; mucB; mucC; |
|       | Biosurfactant                    | rhIA; rhIB;                            |
|       | Iron uptake                      | fptA; pchA; pchB; pchC; pchD; pchE; pchF; pchG; pchH; pchI; pchR; fpvA; fpvD; fpvE; fpvS; |
|       | Pigment                          | phzM; phzS (Pyocyanin)                 |
|       | Protease                         | aprA; lasA; lasB.                      |
|       | Regulation                       | lasI; lasR; rhlL; rhlR;                |
|       | Secretion system                 | xcpP; xcpQ; xcpR; xcpS; xcpT; xcpU; xcpV; xcpW; xcpX; xcpY; xcpZ (Type II secretion system) |
|       | Toxin                            | toxA; exoS; exoT; exoU; exoY; plcH;    |

### Table 3. Comparative analysis against Sphingomonas spp. against major bacterial virulence factors and functions from different pathogenic bacteria.

| BACTERIAL TAXA | MAJOR BACTERIAL VIRULENCE FACTORS (VFS) | SUB VFS | RELATED GENE | E VALUE | IDENT | Sphingomonas GENBANK ACCESSION |
|----------------|------------------------------------------|---------|--------------|---------|-------|--------------------------------|
| Brucella       | Intracellular survival and Immuno-modulatory activity | C[βG (cyclic [β-1,2 glucan] | cgs | 6.00E-59 | 66% | CP006644.1 |
|                | Mannose-1-phosphate guanylyltransferase | manC | 3.00E-09 | 77% | NC_009511.1 |
|                | Phosphoglucomutase                      | pgm | 0 | 74% | NC_009511.1 |
|                | Secretion system                        | VirB type IV secretion system | BMEII0025 | 4.00E-04 | 83% | CP006644.1 |
|                | VirB type IV secretion system            | BMEII0026 | 6.00E-04 | 90% | NC_020561.1 |
|                | VirB type IV secretion system            | BMEII0035 | 4.00E-06 | 72% | NC_020561.1 |
| Legionella     | Adherence                               | Hsp60 | htpB | 2.00E-25 | 64% | NC_020561.1 |
|                | Motility                                | Flagella | flIP | 4.00E-11 | 68% | NC_020561.1 |
|                | Stress protein                          | SodB | 3.00E-05 | 82% | NC_020561.1 |
| Pseudomonas    | Adherence                               | Flagella | ffeQ | 4.00E-65 | 72% | NC_009511.1 |
|                |                                          | ffeF | 5.00E-19 | 67% | NC_009511.1 |
|                |                                          | ffeG | 7.00E-14 | 71% | NC_009511.1 |
|                |                                          | ffeH | 4.00E-29 | 72% | NC_020561.1 |
|                |                                          | ffeI | 6.00E-93 | 68% | NC_020561.1 |
|                |                                          | ffeJ | 6.00E-05 | 89% | NC_020561.1 |
|                |                                          | ffeK | 1.00E-04 | 94% | NC_009511.1 |
|                |                                          | flhA | 2.00E-141 | 69% | NC_020561.1 |
|                |                                          | flhB | 1.00E-18 | 68% | NC_020561.1 |
|                |                                          | flhF | 6.00E-05 | 89% | NC_020561.1 |
|                |                                          | flhC | 1.00E-32 | 72% | NC_009511.1 |
|                |                                          | flhE | 6.00E-04 | 74% | NC_020561.1 |
|                |                                          | flhF | 3.00E-04 | 66% | NC_020561.1 |
|                |                                          | flhG | 8.00E-15 | 69% | NC_020561.1 |

(Continued)
| BACTERIAL TAXA | MAJOR BACTERIAL VIRULENCE FACTORS (VFS) | SUB VFS | RELATED GENE | E VALUE | IDENT | GenBank ACCESSION |
|---------------|------------------------------------------|---------|--------------|---------|-------|------------------|
|               |                                          |         | flh          | 4.00E-05 | 85%   | CP006644.1       |
|               |                                          |         | fliI         | 1.00E-115 | 71%  | NC_009511.1      |
|               |                                          |         | fliN         | 3.00E-16  | 70%  | NC_009511.1      |
|               |                                          |         | fliP         | 9.00E-102 | 75%  | NC_020561.1      |
|               |                                          |         | fliQ         | 2.00E-08  | 84%  | NC_020561.1      |
|               |                                          |         | fliR         | 7.00E-08  | 72%  | NC_020561.1      |
| Pseudomonas   |                                          |         | waaA         | 2.00E-04  | 89%  | CP006644.1       |
|               |                                          |         | waaG         | 4.00E-07  | 76%  | NC_020561.1      |
|               |                                          |         | waaP         | 1.00E-04  | 83%  | NC_020561.1      |
|               |                                          |         | chpA         | 7.00E-26  | 67%  | NC_020561.1      |
|               |                                          |         | chpC         | 8.00E-05  | 82%  | NC_020561.1      |
|               |                                          |         | chpE         | 3.00E-05  | 86%  | CP006644.1       |
|               |                                          |         | fimU         | 3.00E-04  | 93%  | NC_009511.1      |
|               |                                          |         | pilB         | 3.00E-04  | 93%  | NC_009511.1      |
|               |                                          |         | pilD         | 4.00E-05  | 76%  | CP006644.1       |
|               |                                          |         | pilF         | 4.00E-05  | 97%  | CP006644.1       |
|               |                                          |         | pilG         | 2.00E-04  | 80%  | NC_020561.1      |
|               |                                          |         | pilH         | 2.00E-04  | 77%  | NC_009511.1      |
|               |                                          |         | pilI         | 7.00E-06  | 84%  | CP006644.1       |
|               |                                          |         | pilJ         | 5.00E-21  | 76%  | NC_020561.1      |
|               |                                          |         | pilK         | 3.00E-06  | 83%  | NC_020561.1      |
|               |                                          |         | pilN         | 8.00E-04  | 82%  | CP006644.1       |
|               |                                          |         | pilO         | 1.00E-09  | 73%  | NC_009511.1      |
|               |                                          |         | pilR         | 9.00E-54  | 70%  | NC_009511.1      |
|               |                                          |         | pilS         | 6.00E-06  | 74%  | NC_020561.1      |
|               |                                          |         | pilT         | 1.00E-07  | 83%  | NC_009511.1      |
|               |                                          |         | pilU         | 1.00E-12  | 75%  | NC_009511.1      |
|               |                                          |         | pilV         | 2.00E-06  | 72%  | CP006644.1       |
|               |                                          |         | pilW         | 1.00E-05  | 88%  | NC_009511.1      |
| Antiphagocytosis |                                        |         | Alg44        | 7.00E-04  | 85%  | NC_020561.1      |
|               |                                          |         | algA         | 1.00E-21  | 65%  | NC_009511.1      |
|               |                                          |         | algB         | 2.00E-36  | 69%  | NC_009511.1      |
|               |                                          |         | algD         | 2.00E-23  | 72%  | CP006644.1       |
|               |                                          |         | algF         | 3.00E-05  | 91%  | CP006644.1       |
|               |                                          |         | algG         | 8.00E-05  | 85%  | CP006644.1       |
|               |                                          |         | algI         | 1.00E-59  | 67%  | NC_009511.1      |
|               |                                          |         | algJ         | 7.00E-04  | 94%  | NC_009511.1      |
|               |                                          |         | algP         | 1.00E-07  | 80%  | CP006644.1       |
|               |                                          |         | algZ         | 5.00E-05  | 91%  | CP006644.1       |
| Pseudomonas   |                                          |         | rhiA         | 5.00E-04  | 89%  | NC_009511.1      |
| Iron uptake   |                                          |         | fptA         | 6.00E-08  | 86%  | NC_009511.1      |
|               |                                          |         | pchA         | 8.00E-04  | 93%  | NC_009511.1      |
|               |                                          |         | pchB         | 6.00E-04  | 67%  | NC_020561.1      |
|               |                                          |         | pchC         | 1.00E-04  | 80%  | NC_009511.1      |

(Continued)
as adherence, antiphagocytosis, iron uptake, proteases, quorum sensing, and others. With regard to adherence, they shared 20 genes, including flgK with e-value of 1.00E − 04 and identity similarity of 94%, and flgJ with e-value of 6.00E − 05 and identity similarity of 89%. Flagella plays an important role as a virulence factor such as in swimming motility toward the infection site and a role in biofilm formation and other pathogenic adaptations.16–20

Type IV pili play an important role in adherence by assisting the pathogens to attach with their host cells and causing a twitching motility that allows the bacteria to move along the cell surface, and in biofilm formation.17,21–25 Both Sphingomonas spp. and Pseudomonas sp. shared many genes implicated in Type IV pili biogenesis and mechanical function of pili, such as pilF with e-value of 4.00E − 05 and identity similarity of 97%, and pilB with e-value of 3.00E − 04 and identity similarity of 93%.

Furthermore, Sphingomonas spp. and Pseudomonas sp. shared many genes implicated in antiphagocytosis through the production of alginate. They shared 10 alginate genes, including algJ with e-value of 7.00E − 04 and identity similarity of 94%, and algZ with e-value of 5.00E − 05 and identity similarity of 91%. The production of alginate permits pathogenic bacteria to form biofilm and contributes to the persistence of bacteria in the lung by acting as an adhesive, which prevents the bacteria from being expelled from the infection site, and the alginate slime layer makes it more difficult for phagocytes to ingest and kill the bacteria.26–30 Another important bacterial virulence factor shared between Sphingomonas spp. and Pseudomonas sp. is quorum sensing. Sphingomonas spp. showed acquiring of both rhlL and rhlR with e-values of 3.00E − 4 and 5.00E − 9, respectively. These results show that Sphingomonas spp. possesses only rhl system of quorum sensing. While in Pseudomonas sp., quorum sensing consists of two separate but interrelated systems, namely, las and rhl, which are found to regulate the production of multiple virulence factors and are also crucial for proper biofilm formation.31–33

It is also worth mentioning that both Sphingomonas spp. and Pseudomonas sp. share seven genes encoding for xcp secretion system (Type II secretion system), including xcpX and xcpR with e-values of 1.00E − 5 and 3.00E − 174, respectively. The xcp secretion system is found to be responsible for secretion of toxins and enzymes into the extracellular fluid.34,35 It was also observed that both Sphingomonas spp. and Pseudomonas sp.

| GENUS | MAJOR VFS | SUB VFS | RELATED GENE | E VALUE | IDENT | ACCESSION |
|-------|-----------|---------|--------------|---------|-------|-----------|
|       | pchD      |         | 1.00E-09     | 67%     |       | NC_009511.1 |
|       | pchE      |         | 5.00E-12     | 94%     |       | CP006644.1 |
|       | pchF      |         | 9.00E-17     | 79%     |       | CP006644.1 |
|       | pchG      |         | 1.00E-05     | 79%     |       | NC_009511.1 |
|       | pchH      |         | 2.00E-12     | 75%     |       | CP006644.1 |
|       | pchI      |         | 3.00E-16     | 74%     |       | NC_020561.1 |
| Pyoverdine | fpvA    |         | 3.00E-12     | 68%     |       | NC_020561.1 |
| Pigment   | pvdA      |         | 6.00E-05     | 85%     |       | NC_020561.1 |
| Protease  | pvdD      |         | 6.00E-33     | 67%     |       | CP006644.1 |
| Protease  | pvdE      |         | 7.00E-06     | 82%     |       | NC_009511.1 |
| Pyocyanin | phzM      |         | 5.00E-05     | 77%     |       | NC_009511.1 |
| Pyocyanin | phzS      |         | 6.00E-05     | 68%     |       | NC_009511.1 |
| Alkaline protease | aprA |         | 2.00E-12     | 79%     |       | CP006644.1 |
| LasA      | lasA      |         | 2.00E-04     | 86%     |       | NC_020561.1 |
| LasB (Elastase) | lasB |         | 9.00E-04     | 86%     |       | CP006644.1 |
| Quorum sensing | rhlL  |         | 3.00E-04     | 93%     |       | NC_009511.1 |
| Quorum sensing | rhlR  |         | 5.00E-09     | 72%     |       | NC_009511.1 |
| xcp secretion system | xcpQ |         | 2.00E-46     | 70%     |       | NC_009511.1 |
| xcpR      | 3.00E-174  | 72%     | NC_009511.1 |
| xcpS      | 1.00E-44   | 66%     | NC_009511.1 |
| xcpT      | 3.00E-23   | 69%     | NC_009511.1 |
| xcpU      | 2.00E-07   | 78%     | NC_020561.1 |
| xcpW      | 4.00E-04   | 73%     | NC_009511.1 |
| xcpX      | 1.00E-05   | 88%     | NC_020561.1 |
| PLC (Phospholipase C) | plcH |         | 6.00E-33     | 68%     |       | CP006644.1 |
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shared many genes involved in iron uptake using both pyochelin (10 genes) and pyoverdine (4 genes). The pyochelin is effective at promoting iron uptake in \textit{Pseudomonas aeruginosa}, catalyzes the formation of tissue-damaging free radicals, and also binds other transition metals (eg, Mo(IV), Co(II)) with appreciable affinity, and is also implicated in the delivery of both Co(II) and Mo(IV) to \textit{P. aeruginosa} cells.\textsuperscript{36,37} The pyoverdine is effective at acquiring iron from transferrin and lactoferrin. Moreover, pyoverdine is cytotoxic because of its ability to stimulate the production of reactive oxygen species.\textsuperscript{38,39} Interestingly, \textit{Sphingomonas} spp. and \textit{Pseudomonas} sp. shared only one gene responsible for toxin production, namely, plcH that is responsible for degrading the phospholipids surfactant, which functions to reduce the surface tension so that the alveoli do not collapse completely when air leaves them during breathing. It is noteworthy that sphingomyelin (PlcH is a multifunctional enzyme) has been isolated from \textit{Pseudomonas aeruginosa} in 2003.\textsuperscript{40,41}

Our comparative analysis was further extended to search for other bacterial toxins that \textit{Sphingomonas} spp. may acquire. Table 4 shows other toxins that were found to be shared between \textit{Sphingomonas} spp. and \textit{Bordetella pertussis}, which is a Gram-negative species and strictly aerobic coccobacilli. \textit{B. pertussis} is a strict human pathogen causing whooping cough, a highly contagious respiratory disease marked by severe, spasmodic coughing episodes.\textsuperscript{42} It was also observed that \textit{Sphingomonas} spp. contains genes for invasive adenylate cyclase/hemolysin, cyclolysin secretion protein, which is a bifunctional toxin harboring both adenylate cyclase and hemolytic activities, and functions primarily as an anti-inflammatory factor.\textsuperscript{43–45} Moreover, \textit{Sphingomonas} spp. contains genes responsible for pertussis toxin and its secretion system, which assists in the attachment of \textit{B. pertussis} to ciliated respiratory cells, important immunogen and activate cyclic adenosine phosphate (cAMP), histamine sensitizing factor (HSF), lymphocytosis promoting factor (LPF), islet-activating protein (IAP); interferes with leucocyte function; and is hemolytic.\textsuperscript{46,47}

**Conclusion**

Results of this study showed that \textit{Sphingomonas} spp. contains several major virulence factors, mainly resembling those of

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**Table 4.** Suggested \textit{Sphingomonas} spp. toxin information in relation to \textit{Bordetella pertussis} toxins.

| BORDETELLA TOXIN | RELATED GEN | PRODUCT NAME | BORDETELLA PERTUSSIS TOHAMA I | Sphingomonas spp. |
|------------------|-------------|--------------|-------------------------------|------------------|
| Cya (Invasive Adenylate cyclase /haemolysin) | cyaA | Bifunctional hemolysin-adenylate cyclase precursor | 33591934 | 1.00E-21 | 79% | CP006644.1 |
| | cyaD | Cyclolysin secretion protein | 33591936 | 8.00E-04 | 81% | NC_009511.1 |
| | cyaE | Cyclolysin secretion protein | 33591937 | 2.00E-04 | 89% | NC_009511.1 |
| Ptx (Pertussis toxin) | ptxA | Pertussis toxin transport protein | 33594643 | 6.00E-04 | 84% | CP006644.1 |
| | ptxC | Putative bacterial secretion system protein | 33594645 | 3.00E-06 | 88% | NC_009511.1 |
| | ptxD | Putative membrane protein | 33594646 | 8.00E-04 | 78% | CP006644.1 |
| | ptxF | Putative bacterial secretion system protein | 33594649 | 5.00E-04 | 76% | NC_009511.1 |
| | ptxH | Putative bacterial secretion system protein | 33594651 | 6.00E-10 | 71% | CP006644.1 |
| | ptxA | Pertussis toxin subunit 1 precursor | 33594638 | 1.00E-04 | 83% | CP006644.1 |
Pseudomonas sp. Other virulence factors from Legionella sp., Brucella sp., and Bordetella sp. have also been observed. Moreover, the similarity of virulence factors did not correspond to the phylogenetic relationships. These findings suggest horizontal gene transfer of virulence factors rather than sharing a common pathogenic ancestor.

Highlights
- We selected Sphingomonas spp. to test its potentiality for being potential virulent pathogen.
- We tested the phylogenetic relationship of Sphingomonas spp. against known virulent pathogens.
- We screened the presence of various virulent factors from different virulent pathogens in Sphingomonas spp.
- Sphingomonas spp. contains several major virulence factors, mainly resembling those of Pseudomonas sp.

Abbreviations
GDGs: glycosphingolipids.
16S rDNA: 16S ribosomal RNA gene.
NJ: Neighbor-Joining.
MCL: maximum composite likelihood.
tax ID: taxon identity.
cAMP: cyclic adenosine phosphate.
HSF: histamine sensitizing factor.
LPF: lymphocyte promoting factor.
IAP: islet-activating protein.

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Author Contributions
Designed the study methodology, collected information, performed phylogenetic and bioinformatics analyses, and prepared the manuscript: ATMS. Assisted in collecting information, performed phylogenetic and bioinformatics analyses, and prepared the manuscript: SKD, HAB. All authors reviewed and approved of the final manuscript.

Supplementary Material
Supplementary Table 1. This table shows the tested major pathogenic virulence factors.

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