Research Article

Comparative Genomics Reveals Pathogenicity-Related Loci in Shewanella algae

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Shewanella algae is an emerging marine zoonotic pathogen and accounts for considerable mortality and morbidity in compromised hosts. However, there is scarce literature related to the understanding of the genetic background of virulence determinants in S. algae. In this study, we aim to determine the occurrence of common virulence genes in S. algae using whole-genome sequence and comparative genomic analysis. Comparative genomics reveals putative-virulence genes related to bile resistance, chemotaxis, hemolysis, and motility. We detected the existence of hlyA, hlyD, and hlyIII involved in hemolysis. We also found chemotaxis gene cluster cheYZA operon and cheW gene. The results provide insights into the genetic basis underlying pathogenicity in S. algae.

1. Introduction

Shewanella algae is an emerging marine zoonotic pathogen. The organism was first classified in 1990 by Simidu et al. [1], emended by Nozue et al. [2], and described as a Gram-negative, motile bacillus, with hydrogen sulfide production, exhibiting hemolysis on sheep blood agar. S. algae is found in marine environments throughout the world and has been linked with both human and marine animal infections [3, 4]. Currently, there are at least three other Shewanella species found in clinical specimens and S. algae accounts for the majority of isolates from humans [5, 6]. S. algae has also been reported to cause diseases in marine animal, both wild and cultured [7–9]. However, there is scarce literature related to the understanding of the genetic background of virulence determinants in S. algae.

Marine ecosystem consists of a large variety of organisms that impact human health [10]. The advance of sequencing technology allows the identification of determinants in pathogenic microorganisms and has become an important approach to study the fundamental mechanisms of pathogenesis [11, 12]. Comparative genomics further enables the investigation of core elements of pathogenesis factors in great detail [13]. Recently, there have been attempts to use whole-genome sequencing in the study of marine pathogens [14]. Therefore, genomic comparison of the clinical S. algae isolates could provide clues for pathogenic or fitness determinants [15].

The aims of the study were to determine the occurrence of common virulence genes found in S. algae isolates from clinical setting using whole-genome sequence and comparative genomic analysis and to explore the relationship among the tested genomes.

2. Materials and Methods

2.1. Bacterial Strains, Media, and Growth Conditions. S. algae strains ACCC, YHL, and CHL were obtained from various clinical sources (Table 1). Glycerol stock of stored isolates
was grown in trypticase soy agar with 5% sheep blood (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 30°C for 24 hours. Single colonies were inoculated in tryptic soy broth (Becton, Dickinson and Company, Franklin Lakes, NJ). The isolates were preliminarily identified using 16S rRNA gene sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry (bioMérieux, Marcy l’Etoile, France). A part of 16S rRNA gene was amplified using the primers of B27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and U1492R (5’-GGTTACCTTGTTACGACTT-3’) [9, 16]. The nucleotide sequences were aligned, and BLAST search was performed against the GenBank database of the National Center for Biotechnology Information (NCBI) [17].

2.2. Genome Sequencing and Assembly. Nucleic acids were extracted from overnight culture using the QIAGEN Genomic-tip 100G kit and the Genomic DNA Buffer Set (QIAGEN, Paisley, UK) according to the manufacturer’s protocol. The DNA concentrations were measured by Qubit dsDNA HS Assay kit using Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). The DNA sample was sheared, in a microTUBE using Covaris S2 (Covaris, Woburn, MA, USA), into the desired size fragment of the library. The indexed PCR-free library preparation was performed using multiplexed high-throughput sequencing TruSeq DNA Sample Preparation Kit (Illumina) with 2 μg of DNA on the basis of the manufacturer’s protocol. Genome sequencing was performed using paired-end 250 bp sequencing on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA). Raw sequence files were artifact-filtered and trimmed with DUK (http://duk.sourceforge.net/) and FASTX-toolkit fastx_trimmer (https://github.com/agordon/fastx_toolkit), respectively. Assembly was performed with a hybrid approach by ALLPATHS, version R46652 and Velvet version 1.2.07.

2.3. Public Genome Download. Genome sequence of human isolated S. algae MARS 14 was retrieved from the NCBI Genome website (https://www.ncbi.nlm.nih.gov/assembly/GCF_000947195.1/).

2.4. Phylogenetic Analysis Based on Whole-Genome Sequences. Genome-based phylogenetic analysis was performed using pairwise comparison of average nucleotide identity. The whole-genome average nucleotide identity (ANI) was calculated with the use of a modified algorithm [18]. Phylogenetic trees were visualized using MEGA7.

2.5. Annotation and Comparative Genomics. The annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [19] and the DOE-JGI Microbial Genome Annotation Pipeline version 4.10.5 [20]. The prediction was done using Glimmer 3.02 [21]. The non-translated genes were predicted by tRNAscan-SE [22], RNAmmer [23], and RFAM [24]. Functional classification of the predicted genes was carried out using RPSBLAST program v. 2.2.15 [25]. Analysis of the functional annotation was further performed using the Integrated Microbial Genomes & Microbiomes system v.5.0 [26] and the Pathosystems Resource Integration Center [27]. CDS count for these strains was derived. Comparative genome analysis was performed using EDGAR platform (http://edgar.computational.bio) [28]. The core genome and the singletons for the 4 related S. algae genomes were generated for Prokka-annotated genomes using EDGAR (http://edgar.computational.bio). We compared the S. algae genomes using the MUMmer software package [29] together with the Circos visualization engine [30].

3. Results

3.1. Genome Sequencing and Assembly. The genome sequencing consisted of 250 bp paired-end reads, yielding approximately 0.88 Gbp to 1.24 Gbp for each isolate. The de novo assembly of genome sequence data revealed that the number of contigs (>200 bp) varied from 27 to 74 for each genome. The maximum contig size among the genomes was 976,090bp aligned to YHL. The GC content ranged from 52.96% for CHL to 53.08% for ACCC. Table 1 shows the descriptive statistics of the genomic characteristics for the strains in this study. The sequence data were publicly available in NCBI SRA database (accession number: ACCC [LVCY00000000.1], CHL [LVDF00000000.1], and YHL [LVDU00000000.1]).

3.2. Genome-Based Phylogenetic Analysis. The average nucleotide identity (ANI) was calculated and revealed that tested S. algae strains were identical in terms of nucleotide sequences, as shown in Figure 1.
3.3. Comparative Genomics. We constructed a pan-genome dataset using whole-genome sequence of sequenced *S. algae* strains. Figure 2 shows orthologous genes shared among strains and depicts the position and color-coded function of the *S. algae* genes. The numbers of orthologous and strain-specific unique genes are shown in the Venn diagram. Core genome for the *S. algae* strains consists of 1354 coding sequences (Figure 3). The set of unique genes harbored by each strain varies from 335 for *S. algae* YHL to 466 for *S. algae* CHL. Following genome map construction, we conducted genome mapping among the *S. algae* strains in the study. In this comparison, colored arcs indicate regions of high similarity as revealed by the NUCmer script from the MUMmer software package. As shown in Figure 4, the alignment revealed an obvious syntenic relationship in these strains.

3.4. Analysis of Putative-Virulence-Related Genes. As illustrated in Table 2, genes encoded *exbBD*, *galU*, and *htpB* are shared with *S. algae* genomes. Heat shock protein gene *clpP* and hemolysis homologous genes, *hlyA*, *hlyD*, *hlyIII*, and *tolC*, were found in each *S. algae* genome. Gene cluster *cheYZA* operon and *cheW* involved in chemotaxis were detected in all tested *S. algae*. Flagellar gene operons are present in all tested *S. algae* genome.
4. Discussion

*Shewanella algae* has become an emerging marine zoonotic pathogen world-wide [5]. The spectrum of *S. algae* infection is broad with considerable morbidity and mortality in compromised hosts [31, 32]. Thus, understanding genomic characterization of *S. algae* is important for determining molecular epidemiology, understanding its pathogenesis, identifying specific biomarkers, tracing evolution of these strains, and developing control strategy of these pathogens in host...
Figure 3: Comparison of the gene contents of the Shewanella algae in this study, Venn diagram showing the numbers of conserved and strain-specific coding sequences (CDSs).

Figure 4: Genomes mapping between strains in the study. Each colored arc indicates an orthologous match between two species. The color segments in the outer circle are randomly displayed and do not correspond to a particular scheme. A minimum seed match size of 500 bp was used.
| Gene     | locus_tag    | Length | locus_tag    | Length | locus_tag    | Length | locus_tag    | Length |
|----------|--------------|--------|--------------|--------|--------------|--------|--------------|--------|
| hlyA     | BN1227_RS19795 | 443    | AYI97_RS17645 | 443    | AYI82_RS07480 | 443    | AYI77_RS13890 | 443    |
| hlyD     | BN1227_RS18765 | 352    | AYI97_RS03440 | 352    | AYI82_RS05095 | 352    | AYI77_RS05040 | 352    |
| hlyIII   | BN1227_RS10295 | 226    | AYI97_RS09385 | 226    | AYI82_RS06545 | 226    | AYI77_RS04320 | 226    |
| tolC     | AYI97_RS03690  | 466    | AYI97_RS12455 | 466    | AYI82_RS07370 | 466    | AYI77_RS04785 | 466    |
| htpB (groL) | AYI97_RS09385 | 226    | AYI97_RS06545 | 226    | AYI82_RS05095 | 226    | AYI77_RS05040 | 226    |
| galU     | AYI97_RS19990  | 303    | AYI97_RS18495 | 303    | AYI82_RS05620 | 303    | AYI77_RS16730 | 303    |
| exbB     | AYI97_RS02650  | 238    | AYI97_RS04225 | 238    | AYI82_RS07480 | 238    | AYI77_RS13890 | 238    |
| cheY     | AYI97_RS06385  | 127    | AYI97_RS05630 | 127    | AYI82_RS05620 | 127    | AYI77_RS20450 | 127    |
| cheZ     | AYI97_RS06375  | 776    | AYI97_RS05620 | 776    | AYI82_RS05620 | 776    | AYI77_RS20450 | 776    |
| cheA     | AYI97_RS06180  | 696    | AYI97_RS05630 | 696    | AYI82_RS05620 | 696    | AYI77_RS20450 | 696    |
| cheW     | AYI97_RS06350  | 164    | AYI97_RS05595 | 164    | AYI82_RS05620 | 164    | AYI77_RS20950 | 164    |
| clpP      | AYI97_RS05170  | 202    | AYI97_RS17685 | 202    | AYI82_RS05095 | 202    | AYI77_RS14495 | 202    |
| FlgA     | AYI97_RS06595  | 235    | AYI97_RS05050 | 235    | AYI82_RS05050 | 235    | AYI77_RS09380 | 235    |
| FlgB     | AYI97_RS06580  | 132    | AYI97_RS05045 | 132    | AYI82_RS05050 | 132    | AYI77_RS09380 | 132    |
| FlgC     | AYI97_RS06575  | 138    | AYI97_RS05040 | 138    | AYI82_RS05050 | 138    | AYI77_RS09380 | 138    |
| FlgD     | AYI97_RS06570  | 221    | AYI97_RS05035 | 221    | AYI82_RS05050 | 221    | AYI77_RS09380 | 221    |
| Gene | locus_tag      | Length | locus_tag      | Length | locus_tag      | Length | locus_tag      | Length | locus_tag      | Length |
|------|----------------|--------|----------------|--------|----------------|--------|----------------|--------|----------------|--------|
| FlgE | BN1227_RS06915 | 453    | AYI97_RS06565  | 453    | AYI82_RS05810  | 453    | AYI77_RS20820  | 453    |
| FlgF | BN1227_RS06920 | 247    | AYI97_RS06560  | 247    | AYI82_RS05805  | 247    | AYI77_RS20815  | 247    |
| FlgG | BN1227_RS06925 | 263    | AYI97_RS06555  | 263    | AYI82_RS05800  | 263    | AYI77_RS20810  | 263    |
| FlgH | BN1227_RS06930 | 224    | AYI97_RS06550  | 224    | AYI82_RS05805  | 224    | AYI77_RS20815  | 224    |
|      |                |        | AYI82_RS05800  | 223    | AYI77_RS20810  | 223    |
| FlgI | BN1227_RS06935 | 363    | AYI97_RS06545  | 363    | AYI82_RS05800  | 363    | AYI77_RS20810  | 363    |
| FlgJ | BN1227_RS06940 | 336    | AYI97_RS06540  | 336    | AYI82_RS05785  | 336    | AYI77_RS20795  | 336    |
| FlgK | BN1227_RS06945 | 641    | AYI97_RS06535  | 641    | AYI82_RS05780  | 641    | AYI77_RS20790  | 641    |
| FlgL | BN1227_RS06950 | 401    | AYI97_RS06530  | 401    | AYI82_RS05775  | 401    | AYI77_RS20785  | 401    |
| FlgM | BN1227_RS06880 | 106    | AYI97_RS06600  | 106    | AYI82_RS05055  | 94     | AYI77_RS09375  | 94     |
|      | BN1227_RS21265 | 94     | AYI97_RS14315  | 94     | AYI82_RS05845  | 94     | AYI77_RS20855  | 94     |
| FlgN | BN1227_RS06875 | 143    | AYI97_RS06605  | 143    | AYI82_RS05060  | 171    | AYI77_RS09370  | 171    |
| FlgP | BN1227_RS06870 | 155    | AYI97_RS06610  | 155    | AYI82_RS05855  | 171    | AYI77_RS09370  | 171    |
| FlgT | BN1227_RS06860 | 385    | AYI97_RS06620  | 385    | AYI82_RS05865  | 143    | AYI77_RS20860  | 143    |
| FlhA | BN1227_RS07090 | 239    | AYI97_RS06390  | 239    | AYI82_RS04955  | 239    | AYI77_RS20445  | 239    |
| FlhB | BN1227_RS21115 | 236    | AYI97_RS14215  | 236    | AYI82_RS05635  | 236    | AYI77_RS09475  | 236    |
| FlhD | BN1227_RS06970 | 456    | AYI97_RS06510  | 456    | AYI82_RS04980  | 445    | AYI77_RS20325  | 451    |
| FlhE | BN1227_RS21190 | 445    | AYI97_RS14240  | 445    | AYI82_RS05755  | 456    | AYI77_RS20325  | 451    |
| FlhF | BN1227_RS07000 | 110    | AYI97_RS06480  | 110    | AYI82_RS05900  | 111    | AYI77_RS09340  | 111    |
| FlhG | BN1227_RS21300 | 111    | AYI97_RS14350  | 111    | AYI82_RS05725  | 110    | AYI77_RS20355  | 110    |
| FlhH | BN1227_RS07005 | 569    | AYI97_RS06475  | 569    | AYI82_RS05085  | 555    | AYI77_RS09345  | 555    |
| FlhI | BN1227_RS21295 | 555    | AYI97_RS14345  | 555    | AYI82_RS05820  | 555    | AYI77_RS20360  | 569    |
| FlhJ | BN1227_RS07010 | 347    | AYI97_RS06470  | 347    | AYI82_RS05080  | 328    | AYI77_RS09350  | 324    |
| FlhK | BN1227_RS21290 | 328    | AYI97_RS14340  | 328    | AYI82_RS05715  | 328    | AYI77_RS20365  | 347    |
| FlhL | BN1227_RS07015 | 322    | AYI97_RS06465  | 324    | AYI82_RS05710  | 324    | AYI77_RS20370  | 324    |
| FlhM | BN1227_RS07020 | 446    | AYI97_RS06460  | 446    | AYI82_RS05070  | 441    | AYI77_RS09360  | 441    |
| FlhN | BN1227_RS21280 | 441    | AYI97_RS14330  | 441    | AYI82_RS05705  | 446    | AYI77_RS20375  | 446    |
| FlhO | BN1227_RS07025 | 149    | AYI97_RS06455  | 149    | AYI82_RS05700  | 149    | AYI77_RS20380  | 149    |
| FlhP | BN1227_RS07040 | 135    | AYI97_RS06445  | 174    | AYI82_RS04960  | 145    | AYI77_RS11650  | 135    |
| FlhQ | BN1227_RS07035 | 174    | AYI97_RS14220  | 145    | AYI82_RS05690  | 174    | AYI77_RS20390  | 174    |
| FlhR | BN1227_RS21170 | 145    | AYI97_RS17155  | 135    | AYI82_RS09710  | 135    | AYI77_RS20300  | 135    |
Table 2: Continued.

| Gene | locus_tag | Length | locus_tag | Length | locus_tag | Length | locus_tag | Length |
|------|-----------|--------|-----------|--------|-----------|--------|-----------|--------|
| FliM | BN1227_RS07040 | 342 | AY197_RS06440 | 342 | AY182_RS05685 | 342 | AY177_RS18030 | 238 |
|      | BN1227_RS21315 | 300 | AY197_RS14365 | 300 | AY182_RS06430 | 300 | AY177_RS20395 | 342 |
| FliN | BN1227_RS07045 | 126 | AY197_RS06435 | 126 | AY182_RS05110 | 114 | AY177_RS18025 | 114 |
|      | BN1227_RS21320 | 114 | AY197_RS14370 | 114 | AY182_RS05680 | 126 | AY177_RS20400 | 126 |
| FliO | BN1227_RS07050 | 119 | AY197_RS06430 | 119 | AY182_RS05675 | 119 | AY177_RS20405 | 119 |
| FliP | BN1227_RS07055 | 247 | AY197_RS06425 | 247 | AY182_RS05115 | 265 | AY177_RS18020 | 265 |
|      | BN1227_RS21325 | 265 | AY197_RS14375 | 265 | AY182_RS05670 | 247 | AY177_RS20410 | 247 |
| FliQ | BN1227_RS07060 | 89 | AY197_RS06420 | 89 | AY182_RS05120 | 89 | AY177_RS18015 | 89 |
|      | BN1227_RS21330 | 89 | AY197_RS14380 | 89 | AY182_RS05665 | 89 | AY177_RS20415 | 89 |
| FliR | BN1227_RS07065 | 265 | AY197_RS06415 | 265 | AY182_RS05125 | 259 | AY177_RS18010 | 259 |
|      | BN1227_RS21335 | 259 | AY197_RS14385 | 259 | AY182_RS05660 | 265 | AY177_RS20420 | 265 |
| FliS | BN1227_RS06980 | 136 | AY197_RS06500 | 136 | AY182_RS04975 | 126 | AY177_RS09455 | 126 |
|      | BN1227_RS21185 | 126 | AY197_RS14235 | 126 | AY182_RS05745 | 136 | AY177_RS20335 | 136 |
| flhA | BN1227_RS21345 | 692 | AY197_RS14395 | 692 | AY182_RS05135 | 692 | AY177_RS18000 | 692 |
|      | BN1227_RS07075 | 701 | AY197_RS06405 | 701 | AY182_RS05650 | 701 | AY177_RS20430 | 701 |
| flhB | BN1227_RS07140 | 105 | AY197_RS06340 | 105 | AY182_RS05585 | 105 | AY177_RS20960 | 105 |
|      | BN1227_RS21340 | 376 | AY197_RS14390 | 376 | AY182_RS05130 | 376 | AY177_RS18005 | 376 |
|      | BN1227_RS07070 | 378 | AY197_RS06410 | 378 | AY182_RS05655 | 378 | AY177_RS20425 | 378 |
| flhF | BN1227_RS07080 | 458 | AY197_RS06400 | 458 | AY182_RS05645 | 458 | AY177_RS20435 | 458 |
reservoirs. In this study, we investigated the core genetic structure underlying *S. algae* virulence. The pathogenicity and distribution patterns of the *S. algae* strains extended our understanding of their pathogenic potential.

Previous attempts have been made to report the basic features of the genome of *S. algae* from various sources [33, 34]. In the present study, we used comparative genomics to analyze chromosomal sequence of four isolates to determine the common genetic content and organization, unique virulence attributes, and evolutionary relationship with other strains. Whole-genome sequence analysis of *S. algae* detected the presence of chemotaxis gene cluster cheYZA operon that is conserved in the chemotactic bacteria [35]. Chemotaxis is a directed motility in response to concentration gradients of signals. The cheA was demonstrated to be essential for chemotaxis using a two-component pathway [36]. In brief, CheA phosphorylates cheY and then is dephosphorylated by the phosphatase cheZ [37]. Previous studies revealed that CheW and CheA share structural homology and bind to the same site on chemoreceptors [37]. CheW is essential to the activation of CheA and the formation of CheA-CheW complex [38]. Owing to the wide range of *S. algae* habitats, the drivers of its chemotaxis could be very diverse. Previous studies have demonstrated that pathogenic bacteria use chemotaxis to localize reservoirs. Further study would be needed to identify the microenvironments suit for *S. algae* and the trigger of its chemotaxis.

Biliary tract infection is main manifestation of *S. algae* infection, and bile resistance has been noted in pathogenic strains [31]. In the study we also identified genes associated with bile adaption. The *exbBD* gene encodes Ton energy transduction system implicated in the response to bile [39, 40]. We also detected *galU*, *htpB*, and *wecA* involved in bile resistance [41–43]. The results support an earlier genomic study suggesting a common mechanism of bile resistance in *Shewanella*.

Motility is one characteristic of *S. algae* [3]. We identified series of flagellar gene operons in *S. algae* genomes. These flagellar systems are unique and require more study regarding the evolution and organization. Hemolysis is a main pathogenic feature in *S. algae* [44]. The gene *hlyA* encodes RTX pore-forming toxin α-hemolysin, which alters membrane permeability and causes cell lysis in a variety of human and animal hosts [45].

5. Conclusions

In conclusion, this is one of the few studies tracking genetic background of putative virulence-related genes in *S. algae*. Although the number of strains was limited, we highlighted the unique characteristics of core virulence determinants in these strains, as a high level of genomic conservation.

Data Availability

The sequence data are publicly available in NCBI SRA database (accession number: ACCC [LVCY00000000.1], CHL [LVDF00000000.1], and YHL [LVDU00000000.1]).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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