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Original Article

Genomic investigation of emerging zoonotic pathogen *Shewanella xiamenensis*

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Abstract

Objective: *Shewanella xiamenensis* is an emerging zoonotic pathogen commonly found in aquatic ecosystem. Clustered regularly interspaced short palindromic repeats (CRISPR) and (CRISPR)-associated gene systems act as adaptive immune system of prokaryotes. Recently, growing evidence suggested their role in bacterial virulence and resistance. Despite its medical importance, little is known about the genomic characteristics of *S. xiamenensis*. **Materials and Methods:** Strain ZYW6 was isolated from *Epinephelus awoara*. We sequenced the 16S rRNA gene and blast against the GenBank bacterial database. Antibiotic susceptibility tests and interpretation were performed by automatic VITEK 2 system. We extracted the genomic DNA with QIAGEN Genomic-tip 100/G kit and QIAGEN Genomic DNA Buffer Set. Whole-genome shotgun sequencing was performed using the Illumina MiSeq sequencer. To identify the CRISPR-Cas System in the genome of *S. xiamenensis* ZYW6, the Integrated Microbial Genomes and Microbiomes and CRISPRFinder were used. **Results:** We characterized the genome of a *S. xiamenensis* strain. The genome is 4,765,190 bp in length and encodes 4262 open-reading frames. Type I CRISPR-Cas system and serine biosynthesis genes were identified. **Conclusion:** Our results demonstrate the genetic structure of CRISPR-Cas system, l-serine synthesis, and oxacillinase in *S. xiamenensis*. The report of antibiotics resistance genes in the study might indicate a possible reservoir of antimicrobial drug resistance determinants in food animal, resulting in potential infection source. The findings provide insights into the structure and composition of CRISPR-Cas system in *S. xiamenensis* and foundation for future biological validation.

Keywords: *Clustered regularly interspaced short palindromic repeats-Cas system, Serine, Shewanella xiamenensis, Whole-genome sequencing*

Introduction

Emerging infection is a severe threat to global health [1]. Most emerging infections are zoonoses or have zoonotic origins that warrant novel diagnostic and control strategies [2]. *Shewanella xiamenensis* is an emerging zoonotic pathogen first reported in the coastal sea sediment in Xiamen, China [3]. The organism has been reported from aquatic ecosystem worldwide, including wastewater [4,5], freshwater [6], and seawater [7]. It was also found to be the causative organism of human intra-abdominal infection [8] and intestinal colonization [9]. Identification of *S. xiamenensis* is difficult, as illustrated by Zong [8], because 16S rRNA gene sequencing alone is not capable to differentiate *S. xiamenensis* from closely related species. Thus, the true infection rate may be underestimated. The pathogenesis determinants in *S. xiamenensis* are largely unknown.

The rapid advance in sequencing technology changes the study of bacterial infections and leads to the discovery of novel pathogenesis determinants [10]. Genomic sequencing allows the implementation of study of a broad range of pathogenic organisms' characteristics and is applicable on a wide range of pathogens [11]. In many studies of prokaryotes, such as bacterial and archaeal genomes, clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) genes are applied. Early studies demonstrated that the CRISPR-Cas system is an adaptive immune system in prokaryotes. At present, the CRISPR-Cas systems are classified into three distinct types, type I, Type II, and Type III, based on the signature *cas* genes, sequence similarity, and the phylogeny of Cas1 [12]. Type I system consists of six major subtypes (I-A to I-F) which utilize a crRNP to identify targets [13]. Recent studies further suggested its role in virulence and resistance [14]. Earlier study demonstrated L-serine production is an

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important virulent feature in pathogenic bacteria [15]. Although L-serine production was found in *Shewanella* [16,17], little is known about the genetic background.

Despite its medical importance, little is known about the genomic characteristics of *S. xiamenensis*. To identify the CRISPR-Cas systems and genetic background of the L-serine synthesis, we sequenced and analyzed the genome of *S. xiamenensis* strain.

Materials and methods

Strain isolation and antimicrobial susceptibility tests

Strain ZYW6 was isolated from *Epinephelus awoara* on trypticase soy agar with 5% sheep blood (Becton Dickinson, San Jose, CA, USA). The isolate was preliminary identified by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (bioMérieux, Marcy-l'Etoile, France) and by Sanger sequencing of its 16S rRNA gene sequencing [18]. For amplification of the 16S rRNA gene, we used primers with B27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and U1492R (5'-GGTTACCTTGTTACGACTT-3') [19]. Then, we sequenced the PCR product and BLAST against the GenBank bacterial database of the National Center for Biotechnology Information [20]. To confirm the L-serine production in ZYW6, biochemical characterization was performed using API ID 32 GN strips inoculated and read according to the recommendations of the manufacturer (bioMérieux, Marcy-l'Etoile, France). Antibiotic susceptibility tests and interpretation were performed by the VITEK 2 system (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls.

DNA extraction and library preparation

We extracted the genomic DNA with QIAGEN Genomic-tip 100/G kit and QIAGEN Genomic DNA Buffer Set (QIAGEN, Paisley, UK). The library preparation was conducted using a multiplexed high-throughput sequencing TruSeq DNA Sample Preparation Kit (Illumina, San Diego, CA, USA) [19].

Genome sequencing and assembly

We performed sequencing by Illumina MiSeq sequencer using a read length of 250 bp. Consequently, 5,154,704 reads were obtained. There were 1,551,565,904 bp of sequence data. The average read length was 301 bp. The total read depth was 326-fold coverage. The genomic dataset quality was trimmed using duk (http://duk.sourceforge.net/) and the FASTQX-toolkit fastqTrimmer (https://github.com/agordon/fastx_toolkit). Sequencing data were first assembled using Velvet v. 1.2.07 [21] and ALLPATHS v. R46652 [22].

Whole-genome average nucleotide identity analysis

We conducted numerous average nucleotide identity analyses between genomes of ZYT6 and *Shewanella* strains. Pairwise average nucleotide identity between the genome of strains ZYW6 and *Shewanella* strains was calculated using the algorithm proposed by Goris *et al*. [23]. A radial phylogram was constructed using distance matrix computations [24].

Genome annotation

We performed the annotation of the strain ZYW6 according to the NCBI Prokaryotic Genomes Automatic Annotation Pipeline. Besides, functional classification was performed using the RPSBLAST version 2.2.15 [25] and Clusters of Orthologous Groups of proteins databases. To identify the CRISPR-Cas system in the genome of *S. xiamenensis* ZYW6, the integrated microbial genomes and microbiomes [26] and CRISPRFinder [27] were used. All the coding sequences of the genomes were subjected to BLASTn analysis.

We predicated the antibiotic resistance gene (ARG) in the genome with a multiple database-based approach [28]. The comprehensive antibiotic resistance database [29], ResFinder 3.0 [30], and ARG-ANNOT [31]. BLASTn analysis against the JGIs Integrated Microbial Genomes database was used to validate the results [26]. When multiple results are overlapped at the same locus in the genome, the best-aligned virulent factor gene was retained. The candidate virulent genes were predicted using the virulence factor database [32]. The protein sequences of annotated genes were aligned against virulence factor database protein sequences, using BLASTX. All BLASTX results were manually curated based on the consistency of annotations among different databases.

This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number LVDQ00000000. The version described in this paper is version LVDQ00000000.

Results

General genome features

The final assembled genome consisted of forty scaffolds with a total size of 4,765,190 bp (GC content of 46.20%). The maximum contig size was equal to 429,948 bp, and the N50 size was 250,841 bp. Among the 4,262 identified open-reading frames, 4,137 contained protein-coding genes. In addition, 15 rRNA genes (7 5S, 2 16S and 6 23S), 78 tRNA genes, and 5 noncoding RNAs were identified.

Whole-genome average nucleotide identity analysis

The pairwise average nucleotide value between ZYW6 and *Shewanella* strains was calculated. The average nucleotide identity values were 97.6% for ZYW6 and *S. xiamenensis* BC01, 72.3% for *Shewanella algae* YHL, 72.1% for *Shewanella amazonensis* SB2B, 79.9% for *Shewanella baltica* BA175, and 72.5% for *Shewanella loihica* PV-4. As shown in Figure 1, the radial phylogram from alignment fraction analysis revealed an obvious relationship between strains ZYW6 and BC01, which supported the results from the average nucleotide identity analyses.

The clustered regularly interspaced short palindromic repeats-Cas system and *serABC* operon

We identified a Type I-E CRISPR-Cas system, located at position 56,931–65,565 of the genome of *S. algae* ZYW6, containing *cas2* (*ygbF*), *cas1* (*ygbT*), *casE* (*cse3*, *ygcH*), *casD* (*cas5e*, *ygcI*), *casC* (*cse4*, *ygcJ*), *casB* (*cse2*, *ygcK*), *casA* (*cse1*, *ygcL*), and *cas3* (*ygcB*) [Figure 2]. The *S. xiamenensis*

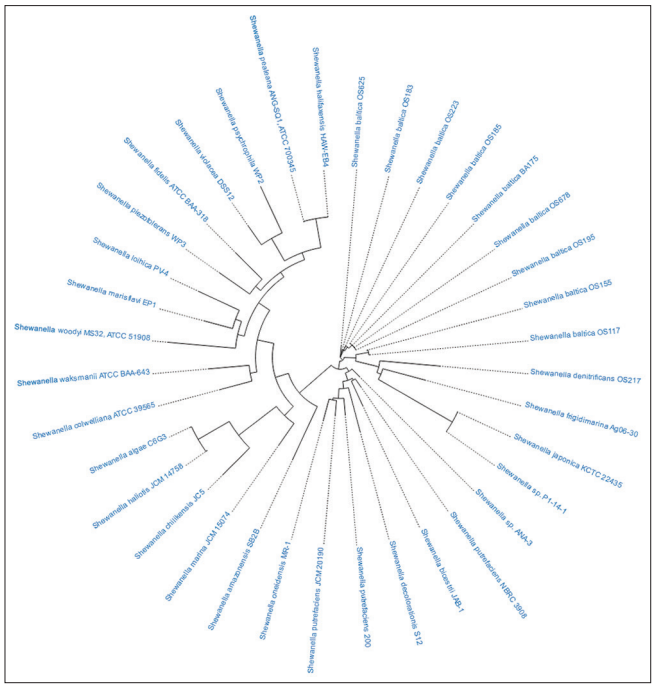

Figure 1: Radial phylogram based on the genomic data from *Shewanella* strains

ZYW6 assimilated l-serine. We detected *serABC* operon which are required for L-serine synthesis from 3-phosphoglycerate.

Resistance profile and related antibiotic resistance gene

The *S. xiamenensis* ZYW6 was susceptible to ampicillin/sulbactam, piperacillin/tazobactam, imipenem, third- and fourth-generation cephalosporin [Table 1]. In our study, bla_{OXA-48} was identified from the genome of *S. xiamenensis* ZYW6.

Discussion

There are increasing reports of *S. xiamenensis* worldwide, raising concern of its pathogenic potential. The emergence of the organism poses threat to both human and animals. Better understanding of the key genomic features is essential for the development of strategies for diagnostic and control measures of this zoonotic infection [33,34]. In the study, we use a genomic approach to characterize the possible genetic background of important antimicrobial resistance and virulence-associated traits [35].

The CRISPR immune system in prokaryotic organisms is a key defense element in neutralizing invading viruses and plasmids [36]. Current evidence revealed that a number of important pathogens harbor Type II CRISPR-Cas system, including *Streptococcus pyogenes*, *Neisseria meningitidis,* and *Haemophilus influenzae* [14]. CRISPR-Cas system-mediated sialylation of the cell envelope was suggested to be one of the underlying mechanisms [37]. The *S. xiamenensis* ZYW6 CRISPR-Cas Type I-E system consists of a gene cluster including *cas2*, *cas1*, the Cascade genes (*casABCDE*), and *cas3*. Complete functionally essential cascade complex is encoded in the system. Type I-E CRISPR-Cas system can be found in many pathogenic bacteria and is associated with their virulence. In enterohemorrhagic *E. coli*, polymorphisms

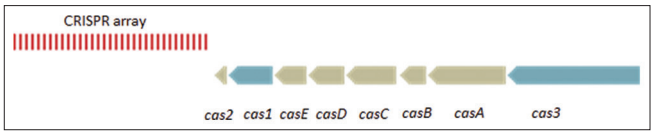

Figure 2: Genetic structure of the clustered regularly interspaced short palindromic repeats-*cas* locus in *Shewanella xiamenensis*

Table 1: Antimicrobial susceptibility profiles of the *Shewanella xiamenensis* ZYW6

Antibiotic(s)	MIC (μg/mL)[a]	Susceptibility[b,c]
Penicillin		
Ampicillin/sulbactam	≤2	S
Piperacillin/tazobactam	≤16/4	S
Cephalosporins		
Ceftriaxone	≤1	S
Ceftazidime	≤1	S
Cefepime	≤1	S
Carbapenems		
Imipenem	0.5	S
Aminoglycosides		
Gentamicin	≤1	S
Amikacin	≤2	S
Fluoroquinolones		
Ciprofloxacin	≤0.25	S
Trimethoprim/sulfamethoxazole	≤20	S

[a]Breakpoint testing only, [b]R: Resistant; S: Susceptible, [c]Susceptible interpretation based on CLSI M100-S27 guidelines for other non-*Enterobacteriaceae*, except for ampicillin/sulbactam, where the U.S. Food and Drug Administration breakpoints were applied. MIC: Minimal inhibitory concentration

in Type I-E CRISPR-Cas system are correlated with the presence of key virulence genes encoding Shiga toxin [38]. Animal study of *Salmonella* and *Campylobacter* also demonstrated that Type I-E CRISPR-Cas system is involved in the pathogenesis [13]. The discovery of Type I-E CRISPR-Cas system in *S. xiamenensis* warrants further studies to elucidate its role in fitness and pathogenesis.

We identified the essential genes of *de novo* L-serine biosynthetic pathway. The biosynthesis of L-serine from D-3-phosphoglycerate includes three reactions, which is catalyzed by D-3-phosphoglycerate dehydrogenase, D-3-phosphoserine aminotransferase, and phosphoserine phosphatase. These are encoded by *serA*, *serC,* and *serB*, respectively. In the study, we identified genes involved in the L-serine biosynthesis pathway, *serA*, *serC*, and *serB*, which is consisted with the biochemical testing results. Overexpressing *serABC* results in improvement of cell growth. Large-scale genomic study has demonstrated the difference of amino acid biosynthesis capabilities between *Staphylococcus aureus* strains and suggested the association with the virulence [39]. Further large-scale pangenomic study is needed to build genome-scale models of *S. xiamenensis*.

Chromosome-encoded carbapenem-hydrolyzing β-lactamase OXA-48 gene was detected in ZYW6. Genes encoding Ambler Class D β-lactamase have been detected in various *Shewanella* species regardless of phenotypic resistance pattern [40]. Our data further suggest that aquaculture-associated *S. xiamenensis*

could be a potential source of Class D β-lactamase gene [41]. Ambler Class D β-Lactamase OXA-48 has been found in human intestinal carriage *S. xiamenensis* [29]. The variants of bla_{OXA} detected in *S. xiamenensis* also included $bla_{OXA-181}$, $bla_{OXA-416}$, $bla_{OXA-204}$, $bla_{OXA-514}$, $bla_{OXA-252}$, and $bla_{OXA-199}$ [42].

Conclusion

Our results demonstrate the genetic structure of CRISPR-Cas system, l-serine synthesis, and oxacillinase in *S. xiamenensis*. The work also highlights the need to conduct large-scale genomic study to fully understand the zoonotic potential and evolutionary changes in *S. xiamenensis*.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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