Complete Genome Sequence of *Stenotrophomonas rhizophila* KC1, a Quorum Sensing–Producing Algicidal Bacterium Isolated from Mangrove *Kandelia candel*

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Abstract

This paper describes the isolation of an algicidal strain, *Stenotrophomonas rhizophila* KC1, from mangrove (*Kandelia candel*) and its genome, which was sequenced using next-generation sequencing technology. The genome is 5.93 Mb with a G+C content of 63.17%. A total of 3,352 functional proteins were assigned according to Kyoto Encyclopedia of Genes and Genomes categories. A total of 11,586 protein-coding genes, 73 transfer RNA genes, and 17 ribosomal RNA genes were obtained. *In silico* genome annotation protocols identified 83 putative quorum sensing (QS) genes, and the algicidal potential of KC1 was related with the QS genes (for example *LuxI-LuxR* genes). Collectively, these data suggest that KC1 may be an antialgal bacterium whose behavior can be modulated by QS signaling. The annotated genome sequence of this strain may represent a valuable tool for studying algae-bacteria interactions and developing microbe-based methods for controlling harmful algae.

Genome Announcement

Harmful algal blooms (HABs) are caused by the rapid proliferation of algae in response to eutrophication and pose a significant threat to both the environment and human health (Anderson 2009; Medlin 2013). HABs can intoxicate humans via the ingestion of contaminated seafood, reduce stocks of wild or cultured fish, and impair tourism (Anderson et al. 2012). The HABs generated by phytoplankton (such as dinoflagellate) has extended the distribution range during the last decades and intensifying the frequency of this events (Anderson et al. 2021). Hence, there is an urgent need to develop methods with which to control algal expansion. In recent years, biological methods have become regarded as good approaches for regulating HABs, largely because such methods may be more targeted and native to marine environments, affording better biosafety for aquatic organisms (Liao et al. 2014; Sengco and Anderson 2004). One such biological method is the use of algicidal bacteria; these have been shown to play an important role in lysing algal cells in aquatic ecosystems (Li et al. 2015; Zhang et al. 2016). Most such bacteria have an algicidal effect that is relatively species-specific. In addition, algaecides that are derived from environmental

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microorganisms are very economical due to the low cost of the source material and because they are ecologically friendly (Zhou et al. 2010).

In this work, environmental samples were collected from the rhizosphere of mangrove *Kandelia candel* on the Shenzhen coast of China. Samples were serially diluted (10^{-1} to 10^{-7}) in sterilized sea water, were spread onto 2216E agar plates (BD Diagnostic Systems), and were then incubated at 30°C for 48 to 72 h. After incubation, colonies were randomly selected according to their morphology, size, and color. The bacterial colony was subcultured on sterile 2216E agar to obtain a pure culture of the bacterial isolate. After this, the bacterial strains were transferred to 1.5-ml EP tubes containing 2216E liquid medium and were then incubated for 24 to 48 h. Algicidal rate was measured according to a previously published method (Zhang et al. 2016). Briefly, it was calculated according to the following equation: algicidal rate (%) = $(N_C - N_E)/N_C \times 10$, where N_C and N_E represent the number of algal cells in the control and experimental groups, respectively. Algal cells were counted after fixing them with Lugol's iodine reagent. The algicidal rate of samples from the algae *Alexandrium tamarense* inoculation system was determined every 6 h. Strain KC1 had the highest rate and, thus, was analyzed further in subsequent experiments. Sequence analysis of the 16S ribosomal RNA (rRNA) gene revealed that it shared 99.8% similarity to the type strain of *Stenotrophomonas rhizophila*. We provisionally named this strain *S. rhizophila* KC1. Currently, 13 assemblies of *S. rhizophila* at different levels are publicly available in GenBank, but only three of them have had their complete genomes sequenced. The three complete genomes were *S. rhizophila* GA1, *S. rhizophila* QL-P4, and *S. rhizophila* JC1. The main biological functions of them were inhibiting algal growth, resistance to heavy metals, and degradation of polyvinyl alcohol (Wei et al. 2017; Yin et al. 2018). Previously, several members of the genus *Stenotrophomonas* have been sequenced, many based on specimens collected from phycosphere environments (Karimi et al. 2020; Yin et al. 2018). To fully understand the molecular information of *S. rhizophila* strains, complete genome assemblies with fewer contigs and improved sequencing quality are essential. Additionally, genome sequencing of this strain will provide greater options for more effective identification of algicidal bacteria, the study of genes of interest (such as those involved in host recognition), and the development of specific molecular markers for population genetic diversity analysis.

Genomic DNA of *S. rhizophila* KC1 was extracted using a genomic DNA extraction kit (MoBio), following the protocols of the manufacturer. The quality of the DNA was checked on agarose gel while the quantity was measured using NanoDrop (Thermo Scientific NanoDrop lite spectrophotometer), which indicated 85 ng per liter. Whole-genome sequencing of the normalized DNA was performed using the Illumina HiSeq 2500 instrument, as described by Glushchenko et al. (2017). The low-quality reads were filtered using Trimmomatic version 1.2.14 (Bolger et al. 2014). *De novo* assembly was performed using CLC Genomics Workbench version 5.1 (CLC Bio) and was trimmed using a minimum Phred quality score of 20, a minimum length of 50 bp, allowing no ambiguous nucleotides, and trimming off some low-quality nucleotides (Bankevich et al. 2012). The reads were assembled with SOAPdenovo (V.2.04) (Li et al. 2010), and sequences were annotated using the RAST annotation server (Aziz et al. 2008). Transfer RNA (tRNA) and rRNA genes were predicted using tRNAscan-SE (Lowe and Eddy 1997) and RNAmmer (Lagesen et al. 2007), respectively. Genes were predicted using Glimmer 3.02 (Delcher et al. 2007) and were annotated by searching against the National Center for Biotechnology Information (NCBI) nonredundant and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Figure 1 presents a circular map of *S. rhizophila* KC1 and Table 1 lists the features of its whole genome. The final whole genome contained 5,234,745 bases and a G+C content of 63.17%. There were 11,586 protein-coding gene sequences with an average size of 928 bp, giving a coding intensity of 88.28%. Assembly of the genome yielded one contig. Further, 134 non-coding RNAs were found in the genome, including 73 tRNAs, 17 rRNAs, and 44 small RNAs (sRNAs). To study homology, we conducted BLAST searches of the NCBI database, which revealed 4,346 coding sequences (CDS) comprising 22 functional clusters of orthologous groups and included 39 KEGG metabolic pathways. Among the CDS, the five most abundant groups in the category of molecular function were amino acid metabolism (342 genes), carbohydrate metabolism (322 genes), translation, ribosomal structure and biogenesis (314 genes), signal transduction mechanisms (242 genes), and transport processes (226 genes), suggesting that two major groups of proteins in this bacterial species are those involved in cellular function and metabolism.

Fig. 1. Circular map for the whole genome of *Stenotrophomonas rhizophila* KC1. From the outside to the center: encoding genes, predicted coding sequences transcribed in the clockwise (or counter clockwise) direction, nondoding RNA, GC percent (%), and GC skew (G+C/G-C) in a 1,000-bp window.

The quorum sensing (QS) signaling pathway usually mediates inter-species communication by producing small extracellular signal molecules (Even-Tov et al. 2016). Previously, Yin et al (2018) reported that algicidal bacterium *S. rhizophila* GA1 has multiple putative QS-like genes located in the upstream position at contig 4. Compared with GA1, there are 83 QS genes in the genome of *S. rhizophila* KC1, which indicated *S. rhizophila* KC1 has more potentiality to regulate its biological behavior under QS. The best-studied QS protein in gram-negative bacteria is *N*-acyl homoserine lactone (AHL). To investigate AHL signaling, we performed *in silico* annotation to identify candidate AI-1 genes based on BLAST analyses. Two genes, encoding a pair of putative *LuxI-LuxR* genes, were subsequently found in the *S. rhizophila* KC1 isolate. Bioinformatics analyses confirmed the presence of essential AHL-related genes. The *LuxI*- and *LuxR*-encoding genes are 509 and 443 bp, respectively, suggesting their potential ability to manipulate cell density via AHL signaling (Martínez et al. 2015). In addition, *LuxI* is frequently detected in species that are closely related to algae-associated bacteria (Zhang et al. 2020). Moreover, a cellulase-coding gene (*cel*) was found downstream of *LuxI-LuxR*; the protein encoded by this gene is believed to contribute to the ability of *S. rhizophila* KC1 to lyse its host. Accordingly, this microorganism is a potential algicidal bacterium with the ability to lyse algae.

In conclusion, the whole-genome sequence dataset generated in this study provides a deeper understanding of the relationships between bacteria and algae under the control of AHLs and may facilitate the development of new microbe-based methods for controlling HABs. The whole-genomics data can supply evidence to the ability of *S. rhizophila* KC1 in enhancing intraspecific communication and algicidal ability, thus making it a potential candidate to control for

Table 1. General genome features of *Stenotrophomonas rhizophila* KC1 and MIGS mandatory information

Item	Description
Sequencing method	Illumina Hiseq 2000
Assembly	SOAPdenovo V.2.04
Genome size	5,929,959 bp
GC content (%)	63.17
Number of contigs	1
Total contig size	5,234,745 bp
Protein-coding genes	11,586
Transfer RNAs	73
Ribosomal RNAs	17
Small RNAs	44
Genes with a predicted function	22
Presumptive quorum-sensing genes	83
Potential <i>LuxI-LuxR</i> encoding site	Contig 1
Encoding- <i>LuxI</i> and <i>LuxR</i> gene lengths	509 and 443 bp

harmful algae. Although the specific substances responsible for this algicidal activity remain unidentified, our data can supply further evidence that AHL has the ability to regulate algicidal behavior. The signal-mediated algicidal system may have potential influence on algal-bacterial interactions and aid in the design of strategies with which to control harmful algae blooms.

Data Availability

The *S. rhizophila* KC1 strain was deposited in the Marine Culture Collection of China with the accession number 1F08522. The complete genome sequence of *S. rhizophila* KC1 is available in GenBank under accession number CP065517.

Author-Recommended Internet Resource

GenBank *S. rhizophila* JC1 chromosome: https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP050062

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