Complete Genome Resource of *Serratia plymuthica* C-1 that Causes Root Rot Disease in Korean Ginseng

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Abstract

Serratia plymuthica C-1, a biocontrol agent, was isolated from soil collected from a mountain forest in Korea. Previous studies have shown that certain strains of *S. plymuthica* cause root rot disease in ginseng. To the best of our knowledge, this is the first report of the sequence of the circular chromosome of *S. plymuthica* C-1, which plays a dual role by causing root rot in ginseng and exhibiting biocontrol activity. The findings of this study will assist in analyzing the genes associated with the pathogenicity and biocontrol properties of *S. plymuthica*.

Serratia plymuthica strains serve as important biological control agents because they produce extracellular enzymes and metabolites that inhibit the growth of various plant pathogens (de Vleeschauwer and Höfte 2007). *Serratia* strains are Gram-negative, rod-shaped, facultative anaerobic bacteria belonging to the family Enterobacteriaceae. Certain strains serve as biocontrol agents and also act as rhizobacteria that promote plant growth (Weise et al. 2014). *S. plymuthica* C-1, which was isolated from forest soil, produces chitinases, which are associated with the biological control potential of biocontrol agents under field conditions (Kim et al. 2008). In field formulations, the chitinolytic activity can help control Phytophthora blight of pepper (Kim et al. 2008). However, certain *S. plymuthica* strains also cause root rot in ginseng (Jung et al. 2018; Kim et al. 2018). Although the complete genome sequences of several biocontrol strains of *Serratia* have been published (Adam et al. 2016; Cleto et al. 2014; Muller et al. 2013; Neupane et al. 2012; Weise et al. 2014), the genome sequence of a plant-pathogenic *Serratia* strain remains unavailable. Here, we report the complete genome sequence of C-1, a strain that is pathogenic on ginseng.

For the genomic analysis, *S. plymuthica* C-1 was cultured for 14 h in nutrient broth (NB) and maintained in 25% glycerol at –80°C. The strain was cultured by transferring the frozen stock onto nutrient agar plates and, after colony formation, a single colony was used to inoculate NB and the culture was subjected to aerobic growth for 2 days at 25°C. The cells were collected by centrifugation, and genomic DNA was extracted using a genomic DNA kit (Macrogen Inc., Daejeon, South Korea) according to the manufacturer's instructions. The quality and quantity of DNA were evaluated using Victor 3 Fluorometry (PerkinElmer, Billerica, MA, U.S.A.) and a Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific Korea Ltd., Seoul, Korea), respectively. Genomic DNA was sequenced at Macrogen Inc. using single-molecule real-time (SMRT) cells with the PacBio RS II SMRT sequencing technology (Pacific Biosciences, Menlo Park, CA, U.S.A.) and a HiSeq X-ten platform

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Keywords

bacterial disease, genomics, ginseng, root rot, *Serratia plymuthica*

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Data availability: This genome project of *Serratia plymuthica* C-1 is indexed in GenBank under the BioProject accession number PRJNA631136 and the Bio Sample ID SAMN14852675. The chromosomal sequence for *S. plymuthica* C-1 is available in GenBank under accession number CP053398.

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Table 1. Genomic features of three biocontrol *Serratia plymuthica* strains

Genomic features	<i>S. plymuthica</i>		
	C-1	S13	3Re4-18
Genome length (bp)	5,695,428	5,467,306	5,439,574
GenBank accession number	CP050260	CP006566	CP012097
Total coding sequence	5,319	4,991	4,943
G+C content (%)	57.80	56.19	56.2
Ribosomal RNA operons	7	7	7
Transfer RNAs	86	84	86
Sequencing platform	PacBio RS II, HiSeq X-ten	Roche/454 GS-FLX	PacBio
Number of contigs	1	1	1
Genome represent	Complete	Complete	Complete
Genome coverage	158X	35X	110X
Source	Forest soil	Styrian oil pumpkin	Potato
Reference	This study	Muller et al. 2013	Adam et al. 2016

(Illumina, San Diego, CA, U.S.A.). The SMRTbell template library of *S. plymuthica* C-1 was constructed according to the steps outlined in the “20kb Template Preparation Using Bluepippin Size-Selection System” protocol using a Covaris g-TUBE device for shearing DNA. The same amplicons were sequenced using the HiSeq X-ten sequencer according to the TruSeq Nano DNA preparation protocol. The software systems were used with default settings, unless specified otherwise. After the raw data were filtered, 140,496 subreads (N_{50} value = 13,119) with an average length of 9,314 bp (total = 1,308,670,812 bp) provided approximately 158-fold sequence coverage. The subreads were assembled de novo using the Canu v1.3 assembler (Koren et al. 2017), and the overlapping regions at both ends of each contig were trimmed to generate unique stretches at both ends using a Circlator (Hunt et al. 2015). These subreads were assembled using the RS hierarchical genome assembly process (HGAP v3.0) and SMRT Portal (v2.3) de novo assembler (Chin et al. 2013.). After de novo assembly, the errors were corrected by generating complete genome contigs with the Illumina reads to compensate for any inaccuracies introduced in the sequencing process. The error-corrected assemblies were further polished using Pilon v1.22 (Walker et al. 2014) with trimmed paired-end reads. After complete genome assembly, BLAST analysis was performed to identify the sequences that exhibited similarity to each scaffold. To assess the completeness of the genome assembly, benchmarking universal single-copy orthologous (BUSCO v3.0) (Seppey et al. 2019) analysis was performed on the basis of evolutionarily informed expectations of gene content from near-universal single-copy orthologs. By default, bacterial_odb9 DB was used for the analysis. The BUSCO analysis yielded 146 complete and single-copy BUSCOs (98.65%), and 2 missing BUSCOs (1.35%) were detected. The genome was annotated using Prokka v1.12b (Seemann 2014) as a pipeline that could perform a series of processes automatically.

The genome of *S. plymuthica* C-1 comprises a single large, circular replicon; its important genomic features are summarized along with those of plant growth-promoting and biocontrol *S. plymuthica* strains in Table 1. The C-1 chromosome has 5,695,428 bp with a 56.15% G+C content and encodes 5,319 proteins, 22 ribosomal RNAs, and 86 transfer RNAs (Table 1). Compared with the genomes of S13 and 3Re4-18, which are two biocontrol and plant-growth-promoting strains, the genome of C-1 is marginally larger in size. Although the *S. plymuthica* C-1 strain is pathogenic to ginseng, gene clusters typically formed by hypersensitive and pathogenicity genes (*hrp*) were not detected in its genome. Additionally, type III-like transporter genes, which are associated with the system that determines which effectors encoded by the *hrp* genes are transferred into the host during pathogenicity, were not detected. However, we detected genes that encode the components characteristic of the type I, II, and IV transport systems. The type strain *S. plymuthica* ATCC 183 (Grimont et al. 1977), isolated from water and known for its ability to produce the follicle-stimulating hormone-binding inhibitor FSH-B1, did not exhibit pathogenic symptoms in carrot or ginseng. *S. marcescens*, a different species belonging to the *Serratia* genus, is pathogenic to both plants and humans (Kirzinger et al. 2011). Genes that encode fimbrial attachment structures, oxidative stress regulators (*oxyR*), or proteins associated with biofilm formation are essential for the virulence of this strain. We intend to conduct studies to identify the genes associated with the virulence of *S. plymuthica* C-1 strain in ginseng and carrot using mutant libraries and gather additional

genetic information, apart from that about chitinase-encoding genes, which plays a central role in the biocontrol potential of this strain.

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