Complete Genome Sequence of the Biocontrol Agent *Pseudomonas chlororaphis* subsp. *aureofaciens* SPS-41

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Abstract

Pseudomonas chlororaphis subsp. *aureofaciens* SPS-41 is a plant growth-promoting rhizobacterium with biocontrol potential that was isolated from the rhizosphere of sweet potato in Xuzhou, Jiangsu Province, China. Our previous study demonstrated that volatile organic compounds (VOCs) produced by SPS-41 inhibited black spot disease fungi *Ceratocystis fimbriata* in postharvest sweet potatoes and a variety of other plant pathogens, and the VOCs also displayed strong nematocidal activity. In order to further explore the application potential of this strain, we here report the complete genome sequence of strain SPS-41. The genome consists of one chromosome (6,757,898 bp) with a G+C content 63.10%, which contains 5,951 coding genes, 67 transfer RNA genes, 16 ribosome RNA genes, and 85 other non-coding RNA genes. No plasmid was detected. The information of the genome will provide resources for studying the biocontrol mechanism of this strain.

Genome Announcement

The rhizosphere of plants is inhabited by a complex microbiome that is essential for the healthy growth and development of plants (Arif et al. 2020). Plant growth-promoting rhizobacteria (PGPRs) produce antibiotics, siderophores, and different hydrolases that can inhibit the occurrence of plant pathogens, which has important application potential in the field of plant biological control and sustainable agriculture (Backer et al. 2018; Bukhat et al. 2020).

Pseudomonas chlororaphis is a kind of PGPR with strong adaptability to various ecological environments. It was recognized as an eco-friendly biocontrol agent, most of which were isolated from the plant rhizosphere (Anderson and Kim 2020). So far, four subspecies of *P. chlororaphis* have been reported, namely, *P. chlororaphis* subsp. *chlororaphis*, *P. chlororaphis* subsp. *aurantiaca*, *P. chlororaphis* subsp. *aureofaciens*, and *P. chlororaphis* subsp. *piscium* (Burr et al. 2010; Peix et al. 2007). *Pseudomonas chlororaphis* has attracted much more attention because of its capacity to produce many secondary metabolites that have the potential for biological control (Arrebola et al. 2019; Guo et al. 2020; Shen et al. 2017), including phenazine 1-carboxamide, phenazine 1-carboxylic acid, pyrrolnitrin, 2-hexyl, 5-propylresorcinol, cyclic peptides, siderophores, hydrogen cyanide, and volatile organic compounds (VOCs) (Anderson and Kim 2020; Biessy et al. 2019; Zhang et al. 2020b).

In our previous study, strain SPS-41, a PGPR, was obtained from the rhizosphere soil of sweet potato. It has been identified as *Pseudomonas chlororaphis* subsp. *aureofaciens* by 16S ribosome RNA (rRNA) gene phylogenetic analysis (Zhang et al. 2020a). Our previous

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Keywords

biocontrol, complete genome, plant growth-promoting rhizobacterium, *Pseudomonas chlororaphis*

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Table 1. General genome features of strain *Pseudomonas chlororaphis* subsp. *aureofaciens* SPS-41

Features	Chromosome
Genome size (bp)	6,757,898
G+C content	63.10%
Protein-coding genes	5,951
Plasmid	0
Ribosome RNAs	16
Transfer RNAs	67
Other non-coding RNA	85
Pseudogene	1
CRISPRs	3
Genomic islands	10
GenBank accession	CP066546

study demonstrated that strain SPS-41 showed broad-spectrum antifungal activity, and the produced VOCs significantly inhibited pathogen *Ceratocystis fimbriata* in postharvest sweet potatoes (Zhang et al. 2019, 2021) and displayed strong nematocidal activity (Zhang et al. 2020a). Here, we report the complete genome sequence of strain *P. chlororaphis* subsp. *aureofaciens* SPS-41 to lay a foundation for further exploring the biological control potential of this strain.

The isolate SPS-41 was cultured in Luria Bertani medium by shaking at 200 rpm overnight at 28°C. Genomic DNA of SPS-41 was extracted, using a standard genomic DNA isolation method, and then, the DNA was transferred to Biomarker Technologies Corporation, Beijing, China to perform the complete genome sequencing by single-molecule real time sequencing based on Oxford Nanopore Technologies.

Sequencing yielded 38,8295 reads including 2,315,521,456 bp, with coverage 310.82× sequence depth. The raw data were filtered and assembled to generate 2,100,541,615 bp of clean data. The filtered subreads were assembled by Canu v1.5 (Koren et al. 2017) to get the draft genome, which was then polished by Pilon 1.22 (Walker et al. 2014) for further correction of the errors of the assembled genome with second generation sequencing data, and high-quality reads were obtained to generate one contig without gaps. Repeated sequence was predicted of the bacterial genome by RepeatMasker (Tarailo-Graovac and Chen 2009). Prodigal was used to retrieve the encoding genes of the assembled genome (Hyatt et al. 2010). Transfer RNA (tRNA) genes were predicted by tRNAscan-SE (version 1.3.1) (Lowe and Eddy 1997), ribosome (rRNA) genes were analyzed by Infernal 1.1 (Nawrocki and Eddy 2013), and other non-coding RNA (ncRNA) other than tRNA and rRNA in the genome were predicted by blasting against the Rfam database (Nawrocki et al. 2015). We compared predicted protein sequences with bacterial protein sequences in the Swiss-Prot database, by GenBlastA (She et al. 2009), to get homologous gene sequences (possible genes), and then, we searched for immature stop codon and frameshift mutations in the gene sequence by GeneWise (Birney et al. 2004) to obtain pseudogenes. The CRISPR recognition tool (Bland et al. 2007) was used to predict CRISPRs in the genome and IslandPath-DIMOB (Langille et al. 2008) was used to predict gene islands in bacterial genomes.

The complete genome of strain SPS-41 contained one circular 6,757,898-bp chromosome with no plasmid. The G+C content was 63.1%, consisting of 5,951 coding genes, 67 tRNA genes, 16 rRNA genes, and 85 other ncRNA genes. The genome of strain SPS-41 contained one pseudogene, three CRISPRs, and 10 genomic islands (Table 1). We also identified 14 putative gene clusters responsible for secondary metabolite biosynthesis by the antiSMASH database (antiSMASH version 5.2.0). Nine of these matched known clusters for the biosynthesis of two pyoverdins, a siderophore, a phenazines, an arylpolyene, a N-acetylglutaminylglutamine amide, a betalactone, a pseudopyronine, an orfamide, and a resorcinol. The remaining clusters were predicted to encode two bacteriocins, one hserlactone, and two nonribosomal peptide synthetase-based compounds.

Pseudomonas chlororaphis subsp. *aureofaciens* SPS-41 exhibited wide-spectrum antifungal and nematocidal activity by producing VOCs. The complete genome sequence could contribute to understanding the genetic and genomic diversities of *P. chlororaphis*. It could also provide opportunities to analyze the biological functions of strain SPS-41. The data obtained in this study have been deposited in GenBank under accession number CP066546.

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