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Complete Genome Sequence Data of a Novel *Streptomyces* sp. Strain A2-16, a Potential Biological Control Agent for Potato Late Blight

e-Xtra*

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Abstract

Streptomyces sp. strain A2-16 was recently isolated from potato root zone soil, and it could inhibit the hyphal growth of *Phytophthora infestans*. The A2-16 genome consisted of one chromosome of 9,765,518 bp and one plasmid of 30,948 bp with GC contents of 70.88% and 68.39%, respectively. A total of 8,518 predicted coding genes, 3 ncRNA,73 tRNA,18 rRNA genes, and 28 secondary metabolite biosynthesis gene clusters were identified. The products of the gene clusters included bioactive polyketides, terpenes, and siderophores, which might contribute to host plants against disease. The average nucleotide identity (ANI) value (82.88–91.41%) among the genome of A2-16 and other *Streptomyces* species suggested it might not belong to any previously sequenced species in the *Streptomyces* genus.

Genome Announcement

Actinobacteria are widely involved in terrestrial and aquatic ecosystems and produce a plethora of secondary metabolites relevant for applications in medicine, agriculture, and biotechnology (Barka et al. 2016; van der Meij et al. 2017). One genus of actinobacteria, *Streptomyces*, has attracted much attention from a biocontrol point of view due to its capacity to produce a large number of antimicrobial compounds (Viaene et al. 2016; Worsley et al. 2020) and its tolerance to massive environmental stress (Liu et al. 2013). Although many *Streptomyces* spp. have been successfully isolated, the commercialization is limited by its field efficacy and stability, as well as strict storage conditions (Colombo et al. 2019; Schlatter et al. 2017). Therefore, it is necessary to discover more new *Streptomyces* spp., especially from the natural habitat, for providing more potential biological control agent (BCA) choices.

Recently, we isolated an actinobacterium A2-16 from root zone soil of potato E-Shu No.5 in Wuxi County of Chongqing City, the main potato-producing area in the southwest of China (patent number: CN112391312-A) (Li et al., *unpublished data*). At present, the strain A2-16 has been deposited in the China general microbiological culture collection center, with the deposit number of CGMCC no. 19402 (http://www.cgmcc.net/). On Gause's No. 1 medium, A2-16 had a cream-colored aerial mycelium and brown with yellow substrate mycelium. Spores derived from broken hyphae were nearly circular. The strain A2-16 can significantly inhibit the hyphal growth and spore germination of *P. infestans* (Feng et al., *unpublished data*), the pathogen causing potato late blight. NCBI blast with 16S rRNA sequence of A2-16 and neighbor-joining (NJ) tree showed it might belong to genus *Streptomyces* (Supplementary Fig. S1). A phylogenetic NJ tree constructed by 31 housekeeping

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genes (*rpoB*, *dnaG*, *frr*, etc.) indicated that A2-16 and *Streptomyces sviceus* are clustered into one group (Supplementary Fig. S2). The average nucleotide identity (ANI) value (82.88–91.41%) among the genomes sequence of A2-16 and nine *Streptomyces* species including *S. sviceus* (GCA_000154965.1) (Yoon et al. 2017) suggested A2-16 might not belong to any previously sequenced species in the *Streptomyces* genus (Supplementary Fig. S3). The ANI value was widely used for traditional bacterial species definition in recent years (>95% intraspecies and <83% intergenus) (Jain et al. 2018; Parks et al. 2020).

The A2-16 strain was cultured in Gause's medium No. 1 for 9 days (Tamreihao et al. 2018) and the strain cells were collected by centrifugation, for genomic DNA extraction with a genomic DNA kit (Omega Bio-Tek Co., Ltd, Guangzhou, China). Based on the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA, U.S.A.) and the HiSeq X-ten platform (Illumina, San Diego, CA, U.S.A.), the sequencing was conducted using the method of single-molecule real-time (SMRT) cells by Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). A SMRTbell library of the strain A2-16 was constructed with a Template Prep Kit v1.0 (Pacific Biosciences) and the base errors of the assembled sequence obtained by the long-read sequences (at least 100× coverage) were corrected by Illumina raw read sequences (about 141× coverage). After the raw data were filtered, a total of 63,780 subreads (N_{50} value = 9,765,518) with an average length of 9,442 bp (total 602,264,600 bp) were obtained. The subreads were assembled de novo using the RS hierarchical genome assembly process (HGAP v3.0) (Chin et al. 2013). The software used in the assembly process mainly included SOAPdenovo2 (Luo et al. 2012) (https://github.com/aquaskyline/SOAPdenovo2) and Canu 2.1 (Koren et al. 2017) (https://canu.readthedocs.io/en/latest/). The Pilon tool of the software (Walker et al. 2014) was used for four to six rounds of short read sequence alignment and base quality correction until all wrong bases were corrected (corrected 81 SNPs; 0 ambiguous bases; corrected 1 small insertion totaling 1 base, 17 small deletions totaling 26 bases). Then, the completeness of genome assembly was assessed by benchmarking universal single-copy orthologous (BUSCO v4.1) (Simão et al. 2015), showing 100% BUSCO completeness. Protein-coding regions, tRNAs, and rRNAs were predicted by rapid annotation subsystem technology NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Overbeek et al. 2014). The gene clusters involved in secondary metabolite production and carbohydrate-active enzymes (CAZymes) were annotated with the antiSMASH 4.0.2 (https://antismash.secondarymetabolites.org/) and CAZymes 6.0 (http://www.cazy.org/) databases, respectively (Cui et al. 2020; Yin et al. 2012).

The final assembled genome of A2-16 consisted of one circular DNA chromosome of 9,765,518 bp with 70.88% GC content and one plasmid of 30,948 bp with GC content of 68.39%, including 8,518 predicted coding genes, 3 ncRNA, 73 tRNA, and 18 rRNA genes. CAZymes database was used to predict carbohydrate-active enzymes. A total of 30 auxiliary activities, 7 carbohydrate-binding modules, 90 carbohydrate esterases, 216 glycoside hydrolases, 14 polysaccharide lyases, and 65 glycosyl transferases were identified (Supplementary Table S1). Those carbohydrate-active enzymes received much attention due to their wide bioactivity, as a biocontrol agent against pathogen and insects (Li et al. 2019; Thadathil and Velappan 2014). AntiSMASH functional analysis found that strain A2-16 has 28 gene clusters involved in secondary metabolite production, including 5 polyketide synthase enzymes (PKS) with 3 canonical types, 5 terpenes, and 4 siderophore gene clusters, etc. (Table 1). Polyketides are widely known in the field of human health and biotechnology due to their wide range of activities including antibacterial, antitumor, and more antagonistic abilities, and their biosynthesis depends on PKS (Caulier et al. 2019). Bacterial terpene is one class of volatile organic compounds (VOCs) that have direct antimicrobial activity and can induce plant defenses or stress tolerance (Reddy et al. 2020; Ryu et al. 2004; Velivelli et al. 2015). For instance, two terpenes (isoprene and monoterpene α-terpineol) produced by *B. subtilis* exhibit antagonistic activities against cyanobacteria and nematodes (Dickschat 2016; Gu et al. 2007). Siderophores can bind metals other than iron and are important mediators of interactions between microbial community members and their eukaryotic hosts (Kramer et al. 2020; Rajkumar et al. 2010). In general, the genome sequence will provide insights into the molecular basis of the biocontrol activity of *Streptomyces* sp. strain A2-16.

Data Availability

The complete genome sequence of *Streptomyces* sp. strain A2-16 has been deposited in NCBI GenBank under accession numbers CP063808 and CP063809 (PRJNA671550 for

Table 1. Secondary metabolite synthesis gene clusters

Cluster	Type	Similar cluster[a]	Similarity to known BGCs (%)
Cluster 1	t2pks[b]	Spore pigment BGC, polyketide	83
Cluster 2	butyrolactone	Neocarzinostatin BGC, polyketide	4
Cluster 3	butyrolactone	Fosfazinomycin BGC, other	7
Cluster 4	terpene	Albaflavenone BGC, terpene	100
Cluster 5	lassopeptide	SSV-2083 BGC, RiPP[c]	50
Cluster 6	siderophore	-	-
Cluster 7	bacteriocin	-	-
Cluster 8	terpene-butyrolactone-t2pks	Xantholipin BGC, polyketide	42
Cluster 9	other	Stenothricin BGC, nrps	13
Cluster 10	siderophore	-	-
Cluster 11	terpene	Hopene BGC, terpene	92
Cluster 12	furan	Colabomycin BGC, polyketide	25
Cluster 13	t2pks	Rabelomycin BGC, polyketide	31
Cluster 14	bacteriocin-lantipeptide	Informatipeptin BGC, RiPP	85
Cluster 15	siderophore	Scabichelin BGC, nrps	20
Cluster 16	bacteriocin	-	-
Cluster 17	bacteriocin	-	-
Cluster 18	terpene	Carotenoid BGC, terpene	63
Cluster 19	t1pks[d]	Maduropeptin BGC, hybrid	3
Cluster 20	melanin	Melanin BGC, other	71
Cluster 21	terpene	-	-
Cluster 22	t3pks	Herboxidiene BGC, polyketide	8
Cluster 23	terpene	Pradimicin BGC, polyketide	7
Cluster 24	other	Pristinamycin BGC, hybrid	2
Cluster 25	ectoine	Ectoine BGC, other	100
Cluster 26	t2pks	Resistomycin BGC, polyketide	88
Cluster 27	melanin	Melanin BGC, other	60
Cluster 28	siderophore	Desferrioxamine_B BGC, other	100

[a] BGC: biosynthetic gene cluster.
[b] t2pks: Type II polyketide synthase enzymes.
[c] RiPP: posttranslationally modified peptides.
[d] t1pks: Type I polyketide synthase enzymes.

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