Complete Genome Sequence Resource for the Endophytic *Burkholderia* sp. Strain MS389 Isolated from a Healthy Soybean Growing Adjacent to Charcoal Rot Disease Patch

Jiayuan Jia, Emerald Ford, Sonya M. Baird, and Shi-En Lu[†]

Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762

Abstract

Burkholderia sp. strain MS389, an endophytic bacterium, was isolated from a healthy soybean plant growing adjacent to a patch of plants affected by charcoal rot disease, caused by the fungal pathogen *Macrophomina phaseolina*. Preliminary studies demonstrated that strain MS389 possesses antimicrobial activities against multiple plant pathogens. *Burkholderia* sp. strain MS389 was found to have three circular chromosomes of 3,563,380 bp, 3,002,449 bp, and 1,180,421 bp in size, respectively. The 7,746,250-bp genome, with 66.73% G+C content, harbors 6,756 protein coding genes in the predicted 6,985 genes. In total, 18 rRNAs, 68 tRNAs, and four ncRNAs were identified and 139 pseudogenes were annotated as well. The findings of this study will provide valuable data to explore the antimicrobial mechanisms of the endophytic bacterial strain.

The bacterial genus *Burkholderia* includes a variety of species and is widespread in the environment (Compant et al. 2008). High genetic versatility and adaptability to various ecological niches are of particular interest and attract increasing attention (O'Sullivan and Mahenthiralingam 2005). *Burkholderia cepacia* complex (Bcc) represents a group of closely related bacteria that are being constantly studied due to the potential of its members to function as plant growth promoters, biocontrol agents, and bioremediators as well as opportunistic pathogens (Chiarini et al. 2006). The Bcc bacteria exist in diverse ecosystems, including soil, water, plants, animals, and humans. Previous studies have shown that plant-associated *Burkholderia* species can act as effective biocontrol agents for plant pathogenic bacteria and fungi, degrade toxic compounds, and fix nitrogen (N₂) (Parke and Gurian-Sherman 2001). The endophytic *Burkholderia* sp. strain MS389, unlike most plant-associated Bcc members that are most typically found in the rhizosphere, was isolated from a healthy soybean plant growing adjacent to a charcoal rot disease patch. This disease is caused by the fungal pathogen *Macrophomina phaseolina*. Plate bioassays revealed that strain MS389 inhibited a broad range of fungal and bacterial plant pathogens (Fig. 1). According to Jin et al. (2020), 116 Bcc strains with high quality complete genomes were reclassified into 36 clusters. Among them, four endophytic bacteria with antagonistic activities were included. Therefore, even though the complete genome sequences of multiple Bcc strains have been published, the genome sequence of an endophytic Bcc strain with strong antimicrobial activity remains comparatively rare. Thus, we report the complete genome sequence of strain MS389, an endophytic strain that may contribute to disease resistance of soybean against the charcoal rot pathogen *M. phaseolina*.

[†]Corresponding author: S.-E. Lu; sl332@msstate.edu

Mention of trade names or commercial products in this publication is solely for the scientific information and does not imply recommendation by the authors.

The author(s) declare no conflict of interest.

Accepted for publication 12 April 2021.

Funding

This study was supported in part by the U.S. Department of Agriculture, National Institute of Food and Agriculture, Hatch project under accession number MIS-4012100, and the Mississippi Soybean Promotion Board, 60-2019.

Keywords

de novo assembly, complete genome, endophyte, soybean, *Burkholderia* spp.

Fig. 1. Antimicrobial activities of strain MS389. A 10 μ l droplet bacterial suspension of MS389 ($OD_{420} = 0.3$) was dropped in the center of agar plates (nutrient broth yeast extract agar: **A**, **B**, and **C**; potato dextrose agar: **D**, **E**, and **F**). After incubation at 28°C for 72 h, the plates were oversprayed with suspensions of indicator bacteria and fungi (**A**, **B**, **C**, **E**, and **F**) or inoculated with agar plugs of the indicator fungus (**D**). The tested indicator bacteria are **A**, *Ralstonia solanacearum* 102; **B**, *Pseudomonas syringae* pv. *syringae* B301D; **C**, *Clavibacter michiganensis* subsp. *michiganensis* 1-07; and the tested indicator fungi include **D**, *Macrophomina phaseolina* 151; **E**, *Cochliobolus heterostrophus* ATCC 48332, and **F**, *Alternaria brassicicola* ATCC 96836. Inhibition zones are indications of antimicrobial activities. The results demonstrate that strain MS389 possesses a broad spectrum of antimicrobial activities against the tested pathogens.

Strain MS389 was isolated in 2014 from an asymptomatic soybean plant that grew adjacent to a charcoal rot disease patch. The plant was washed with tap water, surface disinfected by submerging it in a 10% bleach solution for 5 min, and thoroughly rinsed with sterile water. The plant was cut using a flame-sterilized sterile scalpel and then ground into powder in a sterile blender. Bacterial endophytes were isolated using an ultra-centrifugation-based enrichment method as modified from Ikeda et al. (2009). A portion of the final bacterial suspension was used to prepare serial dilutions. The serial dilutions were plated onto 0.5 $\times$ R2A agar medium. The 0.5 $\times$ R2A agar was prepared by combining 9.2 g Difco R2A Agar (Becton Dickinson #218263) and 9.6 g Difco granulated agar (Becton Dickinson #214530) per liter. Bacterial colonies were picked up and streaked onto nutrient broth yeast extract (NB) agar medium, then incubated at 28°C (Vidaver 1967). Antimicrobial activity of bacterial strain MS389 was identified using the plate bioassay procedure as described previously (Gu et al. 2009). Briefly, cells of strain MS389 were grown overnight on NB liquid medium at 28°C and collected by centrifugation. Bacterial suspension was prepared in sterile distilled water with an optical density (OD) at 420 nm of 0.3. Each of NB and potato dextrose agar (PDA) plates was inoculated with a 10- μ l droplet of the bacterial suspension in the center of the plate and then incubated 72 h at 28°C. The inoculated NB plates were oversprayed with suspensions of the indicator bacteria ($OD_{420} = 0.3$) and PDA plates were oversprayed with suspensions of the indicator fungi ($OD_{420} = 0.3$; D and E) or inoculated with agar plugs of the indicator fungus (F). Inhibition zones are the indication of antimicrobial activities (Fig. 1). Bacterial genomic DNA was extracted from overnight bacterial cultures using Promega Wizard Genomic DNA Purification Kit (Promega). DNA quantity was assessed using a NanoDrop 1000 Spectrophotometer (Thermo Fisher

Table 1. Average nucleotide identity (ANI) (%) between strain MS389 and closely related sequenced strains of the genus *Burkholderia*

<i>Burkholderia</i> species	Genome accession number	ANI (%)
<i>B. contaminans</i> LMG 23361 ^T	GCA_001758385	91.18%
<i>B. stabiliz</i> ATCC BAA-67 ^T	GCA_001742165	91.17%
<i>B. cenocepacia</i> J2315 ^T	GCA_000009485	94.22%
<i>B. pyrrocinia</i> DSM 10685 ^T	GCA_001028665	91.30%
<i>B. cepacia</i> ATCC 25416 ^T	GCA_001411495	91.00%
<i>B. metallica</i> LMG 24068 ^T	GCA_902499065	91.22%
<i>B. seminalis</i> LMG 24067 ^T	GCA_902499165	91.95%
<i>B. puraquae</i> CAMPA 1040 ^T	GCA_002099195	91.24%
<i>B. territorii</i> CCUG 65687 ^T	GCA_008802115	89.79%
<i>B. paludis</i> MSh1T ^T	GCA_000732615	90.71%
<i>B. catarinensis</i> 89 ^T	GCA_001883705	91.63%
<i>B. arboris</i> LMG 24066 ^T	GCA_902499125	92.27%
<i>B. lata</i> 383 ^T	GCA_000012945	91.29%

Scientific, Waltham, MA, U.S.A.). The library preparation and genome sequencing were conducted at the University of Washington PacBio Sequencing Service Center (Seattle, WA) by single-molecule real-time (SMRT) DNA sequencing. To prepare the libraries, the genomic DNA (5 µg) was fragmented into approximately 20-kb pieces using g-TUBE (Covaris), and the resultant fragments were ligated to SMRTbell sequencing adapters using an SMRTbell Template Prep Kit 1.0 (Pacific Biosciences). The library size was measured using Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, U.S.A.) and sequenced using the PacBio Sequel platform. Default parameters were used for all software. In total, 110,742 sub-reads with an N₅₀ value of 13,250 bp and average read length of 7,354 bp were obtained, which provided approximately 102-fold coverage. Genome assembly was carried out using the SMRT Analysis software 2.3.0 with Hierarchical Genome Assembly Process (HGAP) algorithm version 4. The reads were polished using Quiver (Chin et al. 2013) and the overlapping regions of circular sequences were determined by using Gepard (Krumhuk et al. 2007). The genome was automatically annotated using the NCBI's Prokaryotic Annotation Pipeline. Average nucleotide identity (ANI) was used to evaluate genetic relatedness between closely related genomes by using OrthoANI (Lee et al. 2016).

The genome of strain MS389 comprises three unique circular chromosomes totaling 7,746,250 bp in size with 66.73% G+C content. According to the annotation results, chromosome 1 is 3,563,380 bp long, with 66.85% G+C content and 3,205 protein-coding genes; chromosome 2 is 3,002,449 bp long, with 66.60% G+C content and 2,567 protein-coding genes; and chromosome 3 is 1,180,421 bp long, with 66.69% G+C content and 984 protein-coding genes. In total, three chromosomes contain 6,985 genes, with 6,756 protein-coding genes, 139 pseudogenes, 18 rRNAs, 68 tRNAs, and four noncoding RNAs. The strain MS389 genome was compared with the complete genome assemblies downloaded from GenBank for the type strains shown in Table 1. ANI values ranged from 89.79 to 94.22%, with the highest value between strain MS389 and *B. cenocepacia* J2315^T. Richter and Rosselló-Móra (2009) advocate use of ANI 95 to 96% as a benchmark for species demarcation. Since strain MS389 shares the highest similarity (94.22%) with the type strain *B. cenocepacia* J2315^T, its distinct phylogenetic position and potential as a separate species is indicated. Overall, this complete genome will provide valuable data in the endophytic *Burkholderia* spp. and contribute to further understanding antimicrobial mechanisms of the strain MS389.

Data Availability

The genome sequence of *Burkholderia* sp. strain MS389 has been deposited in GenBank in BioProject accession number PRJNA588435 and the BioSample ID SAMN13243640. Sequences of the three chromosomes are deposited in GenBank under accession numbers CP045994, CP045995, and CP045996, respectively.

Literature Cited

- Chiarini, L., Bevivino, A., Dalmastri, C., Tabacchioni, S., and Visca, P. 2006. *Burkholderia cepacia* complex species: health hazards and biotechnological potential. *Trends Microbiol.* 14:277-286.
- Chin, C.-S., Alexander, D. H., Marks, P., Klammer, A. A., Drake, J., Heiner, C., Clum, A., Copeland, A., Huddleston, J., and Eichler, E. E. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* 10:563-569.
- Compant, S., Nowak, J., Coenye, T., Clément, C., and Ait Barka, E. 2008. Diversity and occurrence of *Burkholderia* spp. in the natural environment. *FEMS Microbiol. Rev.* 32:607-626.
- Gu, G., Smith, L., Wang, N., Wang, H., and Lu, S.-E. 2009. Biosynthesis of an antifungal oligopeptide in *Burkholderia contaminans* strain MS14. *Biochem. Biophys. Res. Commun.* 380:328-332.
- Ikeda, S., Kaneko, T., Okubo, T., Rallos, L. E. E., Eda, S., Mitsui, H., Sato, S., Nakamura, Y., Tabata, S., and Minamisawa, K. 2009. Development of a bacterial cell enrichment method and its application to the community analysis in soybean stems. *Microb. Ecol.* 58:703-714.
- Jin, Y., Zhou, J., Zhou, J., Hu, M., Zhang, Q., Kong, N., Ren, H., Liang, L., and Yue, J. 2020. Genome-based classification of *Burkholderia cepacia* complex provides new insight into its taxonomic status. *Biol. Direct* 15:6.
- Krumsiek, J., Arnold, R., and Rattei, T. 2007. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics* 23:1026-1028.
- Lee, I., Kim, Y. O., Park, S.-C., and Chun, J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int. J. Syst. Evol.* 66: 1100-1103.
- O'Sullivan, L. A., and Mahenthiralingam, E. 2005. Biotechnological potential within the genus *Burkholderia*. *Lett. Appl. Microbiol.* 41:8-11.
- Parke, J. L., and Gurian-Sherman, D. 2001. Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annu. Rev. Phytopathol.* 39:225-258.
- Richter, M., and Rosselló-Móra, R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. USA* 106:19126-19131.
- Vidaver, A. M. 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of the carbon source. *Pap. Plant Pathol.* 15:1523-1524.