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GENOME SEQUENCES

Genome Sequence of *Streptomyces cavourensis* 1AS2a, a Rhizobacterium Isolated from the Brazilian Cerrado Biome

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ABSTRACT *Streptomyces cavourensis* strain 1AS2a, isolated from wheat rhizosphere in the Brazilian Neotropical savanna, exhibits strong antimicrobial activities. Its genome comprises 7,600,475 bp with 6,590 open reading frames (ORFs) that reveal 30 biosynthetic gene clusters (BGCs). It provides a genetic basis for further research of the potential of this strain for the production of antimicrobial compounds.

The Brazilian Neotropical savanna (Cerrado) covers more than 20% of Brazil and has been identified as one of the world's biodiversity hotspots. However, although the biodiversity of this biome has not yet been thoroughly explored, recent efforts have highlighted the importance of *Actinobacteria* in the Cerrado (1). Recent studies have described *Actinobacteria* as important producers of compounds with agricultural applications, including antiparasitics, fungicides, larvicides, and nematicides (2). The genus *Streptomyces* is the largest and most prominent group of the phylum *Actinobacteria* with biological applications (3), and almost 1,000 species have been identified from different aquatic and terrestrial environments, mainly in soils and sediments (4).

Streptomyces cavourensis 1AS2a was isolated from a wheat crop in the Brazilian Cerrado, which is located in the middle-west region close to Brasilia DF (15°36′S, 47°42′W). Serial dilutions of the rhizospheric soil were inoculated on International *Streptomyces* Project 2 (ISP-2) medium at 30°C for 5 days; isolation and purification were made considering the morphological similarity of *S. cavourensis* 1AS2a with other *Streptomyces* species (5). Acidic-pH crude extract of *S. cavourensis* 1AS2a was obtained with ethyl acetate solvent (6) and exhibited antimicrobial *in vitro* activity against *Sclerotinia sclerotiorum*, *Micrococcus luteus*, *Escherichia coli*, and *Pythium aphanidermatum*.

Genomic DNA was extracted from a colony pool obtained from *S. cavourensis* 1AS2a that was grown for 3 days at 28°C in ISP-2 broth at 140 rpm using the UltraClean microbial DNA kit (Mo Bio, USA). A draft genome assembly was generated from *S. cavourensis* 1AS2a using paired-end long sequencing with PacBio RS II technology (7) and PacBio P6-C4 chemistry. The library was constructed using BluePippin size selection, with an average fragment of 20 kb (range, 10 to 35 kb). Sequencing was performed using single-molecule real-time (SMRT) cells (8) in an RS II sequencer (UW PacBio Sequencing Services, University of Washington, Seattle, WA). Default parameters were used for all software programs, unless otherwise specified. The raw reads were assembled using Hierarchical Genome Assembly Process (HGAP; version 2.1.1, PacBio data), yielding 7.6 Mb, which combined into 1 contig with 143.1× coverage.

The complete genome of *S. cavourensis* 1AS2a was annotated using Rapid Annotations using Subsystems Technology (RAST) (9, 10). The genome size was determined to be 7,600,475 bp, containing a predicted 6,590 open reading frames (ORFs) and 435 subsystems, with a G+C content of 72.1 mol%. The genome contained 156 genes

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predicted to encode proteins with functions related to stress responses, including cold and heat shock, osmotic, detoxification, and oxidative stress.

In order to identify the BGCs (11) of *S. cavourensis* 1AS2a, additional genome annotation was performed using antiSMASH version 4.2 (12), which identified 30 BGCs, 10 of which matched known clusters for ectoine (13), desferrioxamine B (14), SRO 15-2005 (15), Amfs (16), macrotetrolide (17), bafilomycin (18), SGR_PTMs (19), melanin (20), alkylresorcinol (21), and isorenieratene (22); these had 100% similarity and two clusters encoding griseobactin (23) and coelichelin (24) at >70%. The remaining 18 clusters were predicted to encode polyketide synthase (PKS) types II and III, thiopeptide/PKS1/nonribosomal peptide synthetase (thiopeptide/PKS1/Nrps) hybrid, bacteriocin, aryl polyene, butyrolactone, lantipeptide, thiopeptide, siderophore, and butyrolactone/ectoine hybrid (one of each) proteins, as well as Nrps and terpene (4 of each). The genome sequence information of *S. cavourensis* 1AS2a will facilitate further studies of this strain as a promising source of novel bioactive compound producers, particularly as natural compounds for agricultural application.

Data availability. Raw sequencing data sets have been registered in the NCBI SRA database under accession number SRR8446491. This whole-genome sequencing (WGS) project has been deposited at DDBJ/EMBL/GenBank under the accession number CP024957 and BioProject number PRJNA419149. The version described in this paper is version CP024957.1.

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REFERENCES

1. Quirino BF, Pappas GJ, Tagliaferro AC, Collevatti RG, Neto EL, da Silva M, Bustamante MMC, Krüger RH. 2009. Molecular phylogenetic diversity of bacteria associated with soil of the savanna-like Cerrado vegetation. Microbiol Res 164:59–70. https://doi.org/10.1016/j.micres.2006.12.001.
2. Dhanasekaran D, Thajuddin N, Panneerselvam A. 2010. Herbicidal agents from actinomycetes against selected crop plants and weeds. Nat Prod Res 24:521–529. https://doi.org/10.1080/14786410802299281.
3. Chater KF. 2016. Recent advances in understanding *Streptomyces*. F1000Res 5:2795. https://doi.org/10.12688/f1000research.9534.1.
4. Rey T, Dumas B. 2017. Plenty is no plague: *Streptomyces* symbiosis with crops. Trends Plant Sci 22:30–37. https://doi.org/10.1016/j.tplants.2016.10.008.
5. Shirling EB, Gottlieb D. 1966. Methods for characterization of *Streptomyces* species. Int J Syst Evol Microbiol 16:313–340. https://doi.org/10.1099/00207713-16-3-313.
6. Santos SN, Oliveira LKX, de Melo IS, Velozo ES, Roque M. 2011. Antifungal activity of bacterial strains from the rhizosphere of *Stachytarpheta crassifolia*. Afr J Biotechnol 10:4996–5000.
7. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, deWinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, et al. 2009. Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986.
8. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
9. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226.
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
11. Fischbach M, Voigt CA. 2010. Prokaryotic gene clusters: a rich toolbox for synthetic biology. Biotechnol J 5:1277–1296. https://doi.org/10.1002/biot.201000181.
12. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de los Santos Emmanuel LC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res 45:W36–W41. https://doi.org/10.1093/nar/gkx319.
13. Prabhu J, Schauwecker F, Grammel N, Keller U, Bernhard M. 2004. Functional expression of the ectoine hydroxylase gene (thpD) from *Streptomyces chrysomallus* in *Halomonas elongata*. Appl Environ Microbiol 70:3130–3132. https://doi.org/10.1128/AEM.70.5.3130-3132.2004.
14. Kodani S, Hudson ME, Durrant MC, Buttner MJ, Nodwell JR, Willey JM. 2004. The SapB morphogen is a lantibiotic-like peptide derived from the product of the developmental gene *ramS* in *Streptomyces coelicolor*. Proc Natl Acad Sci U S A 101:11448–11453. https://doi.org/10.1073/pnas.0404220101.
15. Kersten RD, Yang YL, Xu Y, Cimermancic P, Nam SJ, Fenical W, Fischbach MA, Moore BS, Dorrestein PC. 2011. A mass spectrometry-guided genome mining approach for natural product peptidogenomics. Nat Chem Biol 7:794–802. https://doi.org/10.1038/nchembio.684.
16. Ueda K, Oinuma K, Ikeda G, Hosono K, Ohnishi Y, Horinouchi S, Beppu T. 2002. AmfS, an extracellular peptidic morphogen in Streptomyces griseus. J Bacteriol 184:1488–1492. https://doi.org/10.1128/JB.184.5.1488-1492.2002.
17. Smith WC, Xiang L, Shen B. 2000. Genetic localization and molecular characterization of the nonS gene required for macrotetrolide biosynthesis in Streptomyces griseus DSM40695. Antimicrob Agents Chemother 44:1809–1817. https://doi.org/10.1128/AAC.44.7.1809-1817.2000.
18. Zhang W, Fortman JL, Carlson JC, Yan J, Liu Y, Bai F, Guan W, Jia J,

Matainaho T, Sherman DH, Li S. 2013. Characterization of the bafilomycin biosynthetic gene cluster from Streptomyces lohii. Chembiochem 14:301–306. https://doi.org/10.1002/cbic.201200743.
19. Luo Y, Huang H, Liang J, Wang M, Lu L, Shao Z, Cobb RE, Zhao H. 2013. Activation and characterization of a cryptic polycyclic tetramate macrolactam biosynthetic gene cluster. Nat Commun 4:2894. https://doi.org/10.1038/ncomms3894.
20. Funabashi M, Funa N, Horinouchi S. 2008. Phenolic lipids synthesized by type III polyketide synthase confer penicillin resistance on Streptomyces griseus. J Biol Chem 283:13983–13991. https://doi.org/10.1074/jbc.M710461200.
21. Ohnishi Y, Ishikawa J, Hara H, Suzuki H, Ikenoya M, Ikeda H, Yamashita A, Hattori M, Horinouchi S. 2008. Genome sequence of the streptomycin-producing microorganism Streptomyces griseus IFO 13350. J Bacteriol 190:4050–4060. https://doi.org/10.1128/JB.00204-08.
22. Krügel H, Krubasik P, Weber K, Saluz HP, Sandmann G. 1999. Functional analysis of genes from Streptomyces griseus involved in the synthesis of isorenieratene, a carotenoid with aromatic end groups, revealed a novel type of carotenoid desaturase. Biochim Biophys Acta 1439:57–64. https://doi.org/10.1016/S1388-1981(99)00075-X.
23. Patzer SI, Braun V. 2009. Gene cluster involved in the biosynthesis of griseobactin, a catechol-peptide siderophore of Streptomyces sp. ATCC 700974. J Bacteriol 192:426–435. https://doi.org/10.1128/JB.01250-09.
24. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA. 2002. Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 417:141–147. https://doi.org/10.1038/417141a.