

Draft Genome Sequence of an Endophytic *Actinoplanes* Species, Encoding Uncommon *trans*-Acyltransferase Polyketide Synthases

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***Actinoplanes* is an endophytic actinobacterium isolated from the medicinal plant *Amphipterygium adstringens*. The strain draft genome sequence reveals a gene cluster involved in the biosynthesis of a hybrid *trans*-acyltransferase (AT) polyketide, an unconventional bioactive metabolite never reported before in the genus *Actinoplanes*.**

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Endophytic microorganisms from medicinal plants are recognized as a source of novel bioactive natural products (1, 2). *Actinoplanes* sp. was isolated from *Amphipterygium adstringens* (K. Rodríguez-Peña, M. Macías-Rubalcava, L. Rocha Zavaleta, R. Rodríguez-Sanoja, S. Sanchez, unpublished data), a medicinal plant that exhibits a broad range of biological properties, including antitumor, antimicrobial, and anti-inflammatory activities (3–5). Furthermore, *Actinoplanes* spp. are a group of actinobacteria known for producing pharmaceutically important compounds, such as the antimicrobial teicoplanin (6, 7) and anticancer (8) and antidiabetic drugs (9), emerging as an important genus to search for novel specialized metabolites by genome mining (9, 10).

The genome sequencing of *Actinoplanes* sp. was performed by the BaseClear Company (The Netherlands), using the Illumina HiSeq 2500 system. Approximately 508 Mb of raw data of 125 bp-long paired-end reads were generated, resulting in 54-fold coverage, with average quality scores (Phred) of 33.7. *De novo* assembly was performed using SPAdes version 3.5.0 (11). The resulting contigs were ordered with Ragout version 0.2 (12) using *Actinoplanes* sp. strain N902-109 (GenBank accession no. PRJNA198760) as the reference genome. The assembly was improved by remapping reads using Pilon version 1.12 (13). The draft genome sequence of 7,752,284 bp comprises 24 scaffolds, 7,161 protein-coding sequences, 67 RNAs, and a G+C content of 68.9%. Sequence analysis was performed by combining antiSMASH version 3.0.4 (14) and the PRISM genomic analysis platform (15). The analysis predicted 16 gene clusters coding for enzymes involved in the biosynthesis of secondary metabolites, including bacteriocins, type 1 and 3 polyketide synthases (PKS), and nonribosomal peptide synthases (NRPS). Remarkably, one gene cluster for the biosynthesis of an uncommon hybrid *trans*-acyltransferase (AT) polyketide was also found. *trans*-AT-PKS are an unconventional class of multimodular enzymes never reported before in *Actinoplanes*. These enzymes are frequently found in

bacterial symbionts (16–18), which lack acyltransferase (AT) domains but instead receive its acyl precursor by a free-standing AT (18, 19). Sequence analysis performed using 2metDB, Pfam, and UniProt (20, 21) revealed a hybrid NRPS-t2PKS-*trans*-AT-PKS-t1PKS cluster, suggesting a highly complex biosynthetic pathway. The BLAST sequence analysis of the NRPS-t2PKS-*trans*-AT-PKS-t1PKS enzymes indicates a low level (<40%) of sequence identity compared to similar modules coded by the genomes of the *Paenibacillaceae* and *Peptococcaceae* families and the genera *Bacillus* and *Streptomyces*. These findings strongly support the presence of a novel hybrid *trans*-AT-PKS gene cluster. Since antitumor (22, 23) and antimicrobial activities (24) have been reported for *trans*-AT polyketides, the potential of this endophytic actinobacterium as a source of novel natural bioactive compounds is considerable.

Nucleotide sequence accession numbers. The whole-genome shotgun (WGS) project of *Actinoplanes* sp. was deposited at DDBJ/EMBL/GenBank under the WGS project accession no. LOJP00000000. The version described in this paper is the first version, LOJP01000000.

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REFERENCES

- Alvin A, Miller KI, Neilan BA. 2014. Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. *Microbiol Res* 169:483–495. <http://dx.doi.org/10.1016/j.micres.2013.12.009>.
- Christina A, Christopher V, Bhore SJ. 2013. Endophytic bacteria as a source of novel antibiotics: an overview. *Pharmacogn Rev* 7:11–16. <http://dx.doi.org/10.4103/0973-7847.112833>.
- Castillo-Juárez I, García-Conrteras R, Velázquez-Guadarrama N, Soto-

- Hernández M, Martínez-Vázquez M. 2013. *Amphytergyium Adstringens* anacardic acid mixture inhibits quorum sensing-controlled virulence factors of *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. Arch Med Res 44:488–494. <http://dx.doi.org/10.1016/j.arcmed.2013.10.004>.
4. Ramírez-León A, Barajas-Martínez H, Flores-Torales E, Orozco-Barocio A. 2013. Immunostimulating effect of aqueous extract of *Amphytergyium adstringens* on immune cellular response in immunosuppressed mice. Afr J Tradit Complement Altern Med 10:35–39.
 5. Navarrete A, Oliva I, Sánchez-Mendoza ME, Arrieta J, Cruz-Antonio L, Castañeda-Hernández G. 2005. Gastroprotection and effect of the simultaneous administration of cuachalalate (*Amphytergyium adstringens*) on the pharmacokinetics and anti-inflammatory activity of diclofenac in rats. J Pharm Pharmacol 57:1629–1636. <http://dx.doi.org/10.1211/jpp.57.12.0013>.
 6. Chu M, Truumees I, Mierzwa R, Terracciano J, Patel M, Das PR, Puar MS, Chan T. 1998. A new potent antifungal agent from *Actinoplanes* sp. Tetrahedron Lett 39:7649–7652. [http://dx.doi.org/10.1016/S0040-4039\(98\)01696-7](http://dx.doi.org/10.1016/S0040-4039(98)01696-7).
 7. Jung H-M, Jeya M, Kim S-Y, Moon H-J, Kumar Singh R, Zhang Y-W, Lee J-K. 2009. Biosynthesis, biotechnological production, and application of teicoplanin: current state and perspectives. Appl Microbiol Biotechnol 84:417–428. <http://dx.doi.org/10.1007/s00253-009-2107-4>.
 8. Breyer S, Effenberger-Neidnicht K, Knauer S, Schobert R. 2011. Synthesis, anticancer activity, and iron affinity of the *Actinoplanes* metabolite 7,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione. Bioorg Med Chem 19:1264–1267. <http://dx.doi.org/10.1016/j.bmc.2010.12.012>.
 9. Schwientek P, Szczepanowski R, Rückert C, Kalinowski J, Klein A, Selber K, Wehmeier UF, Stoye J, Pühler A. 2012. The complete genome sequence of the acarbose producer *Actinoplanes* sp. SE50/110. BMC Genomics 13:112. <http://dx.doi.org/10.1186/1471-2164-13-112>.
 10. Yim G, Kalan L, Koteva K, Thaker MN, Waglechner N, Tang I, Wright GD. 2014. Harnessing the synthetic capabilities of glycopeptide antibiotic tailoring enzymes: characterization of the UK-68,597 biosynthetic cluster. ChemBiochem 15:2613–2623. <http://dx.doi.org/10.1002/cbic.201402179>.
 11. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477.
 12. Kolmogorov M, Raney B, Paten B, Pham S. 2014. Ragout-a reference-assisted assembly tool for bacterial genomes. Bioinformatics 30:i302–i309. <http://dx.doi.org/10.1093/bioinformatics/btu280>.
 13. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. <http://dx.doi.org/10.1371/journal.pone.0112963>.
 14. Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T. 2013. antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res 41:W204–W212. <http://dx.doi.org/10.1093/nar/gkt449>.
 15. Skinnider MA, Dejong CA, Rees PN, Johnston CW, Li H, Webster ALH, Wyatt MA, Magarvey NA. 2015. Genomes to natural products PRediction Informatics for Secondary Metabolomes (PRISM). Nucleic Acids Res 43:9645–9662. <http://dx.doi.org/10.1093/nar/gkv1012>.
 16. Trindade-Silva AE, Rua CPJ, Andrade BGN, Vicente ACP, Silva GGZ, Berlinck RGS, Thompson FL. 2013. Polyketide synthase gene diversity within the microbiome of the sponge *Arenosclera brasiliensis*, endemic to the southern Atlantic Ocean. Appl Environ Microbiol 79:1598–1605. <http://dx.doi.org/10.1128/AEM.03354-12>.
 17. Kwan JC, Schmidt EW. 2012. Cleaning up polyketide synthases. Chem Biol 19:309–311. <http://dx.doi.org/10.1016/j.chembiol.2012.03.003>.
 18. Piel J. 2010. Biosynthesis of polyketides by *trans*-AT polyketide synthases. Nat Prod Rep 27:996–1047. <http://dx.doi.org/10.1039/b816430b>.
 19. Dunn BJ, Watts KR, Robbins T, Cane DE, Khosla C. 2014. Comparative analysis of the substrate specificity of *trans*- versus *cis*-acyltransferases of assembly line polyketide synthases. Biochemistry 53:3796–3806. <http://dx.doi.org/10.1021/bi5004316>.
 20. Bachmann BO, Ravel J. 2009. Methods for *in silico* prediction of microbial polyketide and nonribosomal peptide biosynthetic pathways from DNA sequence data. Methods Enzymol 458:181–217. [http://dx.doi.org/10.1016/S0076-6879\(09\)04808-3](http://dx.doi.org/10.1016/S0076-6879(09)04808-3).
 21. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M. 2014. Pfam: the protein families database. Nucleic Acids Res 42:D222–D230. <http://dx.doi.org/10.1093/nar/gkt1223>.
 22. Pulsawat N, Kitani S, Nihira T. 2007. Characterization of biosynthetic gene cluster for the production of virginiamycin M, a streptogramin type A antibiotic, in *Streptomyces virginiae*. Gene 393:31–42. <http://dx.doi.org/10.1016/j.gene.2006.12.035>.
 23. Tang GL, Cheng YQ, Shen B. 2004. Architectural complexity for a hybrid polyketide synthase and nonribosomal peptide synthetase. Chem Biol 11:33–45. <http://dx.doi.org/10.1016/j.chembiol.2003.12.014>.
 24. Tatsuno S, Arakawa K, Kinashi H. 2007. Analysis of modular-iterative mixed biosynthesis of lankacidin by heterologous expression and gene fusion. J Antibiot 60:700–708. <http://dx.doi.org/10.1038/ja.2007.90>.