



New Genome Sequence of an *Echinacea purpurea* Endophyte, *Arthrobacter* sp. Strain EpSL27, Able To Inhibit Human-Opportunistic Pathogens

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ABSTRACT We announce here the draft genome sequence of *Arthrobacter* sp. strain EpSL27, isolated from the stem and leaves of the medicinal plant *Echinacea purpurea* and able to inhibit human-pathogenic bacterial strains. The genome sequencing of this strain may lead to the identification of genes involved in the production of antimicrobial molecules.

Medicinal plants are well known and have been largely explored for centuries for their therapeutic properties (1). What is little known is that their therapeutic potential could be related to endophytic microorganisms inhabiting their tissues (2). Many bioactive molecules have been already extracted from endophytic bacteria (3). The promising potential of such organisms has led to the characterization of endophytic bacterial communities from medicinal plants, which are poorly known. Endophytic and rhizospheric bacterial communities from the medicinal plants *Echinacea purpurea* and *Echinacea angustifolia* have been characterized, highlighting the specific composition of such communities within plants' compartments (4). *Arthrobacter* sp. strain EpSL27, extracted from the stem and leaves of *E. purpurea*, has been evidenced as being resistant to a high level of oxidative stress (20 mM H₂O₂) and is able to degrade diesel fuel. Among such notable biotechnological potentialities, *Arthrobacter* sp. EpSL27 has also been found to show strong inhibition activity toward human-pathogenic bacteria from the *Burkholderia cepacia* complex (5), which are multidrug-resistant organisms able to induce serious infections in immunocompromised patients.

The intriguing information obtained by the above-cited analyses led to whole sequencing of the strain genome.

Arthrobacter sp. EpSL27 genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (6), and its authenticity has been confirmed by 16S rRNA gene sequencing. Whole-genome shotgun sequencing was performed with a 2 × 300-bp paired-end approach using the MiSeq sequencing system (Illumina, Inc., San Diego, CA). The FastQC software package version 0.52 (7) was used to evaluate the quality of the obtained read pairs, and poor-quality bases were removed using Streaming-Trim (8). Assembly was performed using the SPAdes 3.5 software (9), with k-mer lengths of 21, 33, and 55, generating 21 contigs. Those having a length shorter than 200 nucleotides were removed and the others launched for scaffolding through Medusa software (10), using the following genomes as references: *Arthrobacter arilaitensis* Re117 (11), *Arthrobacter* Rue61a (12), *Arthrobacter* sp. strain FB24 (13), *Arthrobacter*

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aurantiacus TC1 (14), and *Arthrobacter chlorophenicus* A6. The resulting scaffolds were then annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAAP) (15). The final version of the *Arthrobacter* sp. EpSL27 draft genome consists of 8 scaffolds, and its total length is 4,176,054 bp, with a coverage of 215.0×. The G+C content is about 67.8%, which reflects the characteristic high G+C content of the genus. The *Arthrobacter* sp. EpSL27 genome harbors 3,758 genes, 3,610 of which are protein-coding genes, 66 are RNA-coding genes (5 5S rRNA, 1 23S rRNA, 1 16S rRNA, 50 tRNAs, and 9 noncoding RNA [ncRNA]), and 91 are pseudogenes.

The EpSL27 genome was analyzed using CARD (16) for the presence of genes conferring antibiotic resistance. The analysis has evidenced genes putatively involved in specific antibiotic resistance to isoniazid (*Mycobacterium tuberculosis kasA* mutant), fluoroquinolones (*mfd*), aminocoumarin (*Streptomyces rishiriensis parY* mutant), rifamycin (*rphB*), mupirocin (*Bifidobacterium* intrinsic *ileS*), and fosfomycin (*Chlamydia trachomatis* intrinsic *murA*). antiSMASH (17) analysis for secondary metabolites with antimicrobial activities was also performed, revealing the presence of 5 clusters, with one cluster encoding nonribosomal peptide synthetase (NRPS), one cluster encoding type 3 polyketide synthase (T3pks), and another three clusters with an unspecified reference.

Accession number(s). The whole-genome shotgun project has been deposited at NCBI whole-genome sequencing (WGS) database under accession number [LNUT00000000](https://www.ncbi.nlm.nih.gov/assembly/LNUT00000000/), and the version reported in this work is version LNUT00000000.1.

REFERENCES

1. Staub PO, Casu L, Leonti M. 2016. Back to the roots: a quantitative survey of herbal drugs in Dioscorides' de materia medica (ex Matthioli, 1568). *Phytomedicine* 23:1043–1052. <https://doi.org/10.1016/j.phymed.2016.06.016>.
2. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. 2008. Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett* 278:1–9. <https://doi.org/10.1111/j.1574-6968.2007.00918.x>.
3. Shweta S, Bindu JH, Raghu J, Suma HK, Manjunatha BL, Kumara PM, Ravikanth G, Nataraja KN, Ganeshiah KN, Uma Shaanker R. 2013. Isolation of endophytic bacteria producing the anti-cancer alkaloid camptothecin from *Miquelia dentata* Bedd. (Icacinaceae). *Phytomedicine* 20: 913–917. <https://doi.org/10.1016/j.phymed.2013.04.004>.
4. Chiellini C, Maida I, Emiliani G, Mengoni A, Mocali S, Fabiani A, Biffi S, Maggini V, Gori L, Vannacci A, Gallo E, Firenzuoli F, Fani R. 2014. Endophytic and rhizospheric bacterial communities isolated from the medicinal plants *Echinacea purpurea* and *Echinacea angustifolia*. *Int Microbiol* 17:165–174. <https://doi.org/10.2436/20.1501.01.219>.
5. Chiellini C, Maida I, Maggini V, Bosi E, Mocali S, Emiliani G, Perrin E, Firenzuoli F, Mengoni A, Fani R. 2017. Preliminary data on antibacterial activity of *Echinacea purpurea*-associated bacterial communities against *Burkholderia cepacia* complex strains, opportunistic pathogens of cystic fibrosis patients. *Microbiol Res* 196:34–43. <https://doi.org/10.1016/j.micres.2016.12.001>.
6. Perrin E, Fondi M, Maida I, Mengoni A, Chiellini C, Mocali S, Cocchi P, Campana S, Taccetti G, Vanechoutte M, Fani R. 2015. Genomes analysis and bacteria identification: the use of overlapping genes as molecular markers. *J Microbiol Methods* 117:108–112. <https://doi.org/10.1016/j.mimet.2015.07.025>.
7. Kunde-Ramamoorthy G, Coarfa C, Laritsky E, Kessler NJ, Harris RA, Xu M, Chen R, Shen L, Milosavljevic A, Waterland RA. 2014. Comparison and quantitative verification of mapping algorithms for whole-genome bisulfite sequencing. *Nucleic Acids Res* 42:e43. <https://doi.org/10.1093/nar/gkt1325>.
8. Bacci G, Bazzicalupo M, Benedetti A, Mengoni A. 2014. StreamingTrim 1.0: a Java software for dynamic trimming of 16S rRNA sequence data from metagenetic studies. *Mol Ecol Resour* 14:426–434. <https://doi.org/10.1111/1755-0998.12187>.
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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. <https://doi.org/10.1089/cmb.2012.0021>.
10. Bosi E, Donati B, Galardini M, Brunetti S, Sagot MF, Lió P, Crescenzi P, Fani R, Fondi M. 2015. Medusa: a multi-draft based scaffolder. *Bioinformatics* 31:2443–2451. <https://doi.org/10.1093/bioinformatics/btv171>.
11. Monnet C, Loux V, Gibrat JF, Spinnler E, Barbe V, Vacherie B, Gavory F, Gourbeyre E, Siguier P, Chandler M, Elleuch R, Irlinger F, Vallaeyts T. 2010. The *Arthrobacter arilaitensis* Re117 genome sequence reveals its genetic adaptation to the surface of cheese. *PLoS One* 5:e15489. <https://doi.org/10.1371/journal.pone.0015489>.
12. Niewerth H, Schultes J, Parschat K, Kiefer P, Vorholt JA, Daniel R, Fetzner S. 2012. Complete genome sequence and metabolic potential of the quinaldine-degrading bacterium *Arthrobacter* sp. Rue61a. *BMC Genomics* 13:534. <https://doi.org/10.1186/1471-2164-13-534>.
13. Nakatsu CH, Barabote R, Thompson S, Bruce D, Detter C, Brettin T, Han C, Beasley F, Chen W, Konopka A, Xie G. 2013. Complete genome sequence of *Arthrobacter* sp. strain FB24. *Stand Genomic Sci* 9:106–116. <https://doi.org/10.4056/sigs.4438185>.
14. Mongodin EF, Shapir N, Daugherty SC, DeBoy RT, Emerson JB, Shvartzbeyn A, Radune D, Vamathevan J, Riggs F, Grinberg V, Khouri H, Wackett LP, Nelson KE, Sadowsky MJ. 2006. Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. *PLoS Genet* 2:e214. <https://doi.org/10.1371/journal.pgen.0020214>.
15. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. *OMICS* 12:137–141. <https://doi.org/10.1089/omi.2008.0017>.
16. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJV, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD. 2013. The Comprehensive Antibiotic Resistance Database. *Antimicrob Agents Chemother* 57:3348–3357. <https://doi.org/10.1128/AAC.00419-13>.
17. Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43:W237–W243. <https://doi.org/10.1093/nar/gkv437>.