

Draft Genome Sequence of *Methylobacterium* sp. Strain L2-4, a Leaf-Associated Endophytic N-Fixing Bacterium Isolated from *Jatropha curcas* L.

Munusamy Madhaiyan, Kam Lock Chan, Lianghui Ji

Biomaterials and Biocatalysts Group, Temasek Life Sciences Laboratory, National University of Singapore, Singapore

***Methylobacterium* sp. strain L2-4 is an efficient nitrogen-fixing leaf colonizer of biofuel crop *Jatropha curcas*. This strain is able to greatly improve the growth and seed yield of *Jatropha curcas* and is the second reported genome sequence of plant growth-promoting bacteria isolated from *Jatropha curcas*.**

Received 3 November 2014 Accepted 7 November 2014 Published 11 December 2014

Citation Madhaiyan M, Chan KL, Ji L. 2014. Draft genome sequence of *Methylobacterium* sp. strain L2-4, a leaf-associated endophytic N-fixing bacterium isolated from *Jatropha curcas* L. *Genome Announc.* 2(6):e01306-14. doi:10.1128/genomeA.01306-14.

Copyright © 2014 Madhaiyan et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Lianghui Ji, [jih@tll.org.sg](mailto:jlh@tll.org.sg).

As biofuel crop *Jatropha curcas* is targeted to marginal land where soil nutrient is low, the requirement for nitrogen fertilizer will be higher than other crops. The use of plant growth-promoting bacteria is a promising approach to improve productivity and the green index of *Jatropha* biodiesel (1–3). *Methylobacterium* species are abundant on plant leaf tissues as endophytes or epiphytes on leaves (4–11) and exert a positive effect on the growth and development of plants by playing a role in seed germination and root development, drought tolerance, growth promotion, and increasing the yield of agricultural plants (12, 13). Previously, some *Methylobacterium* strains have been found in symbiotic association with *Crotalaria* and *Lotononis*, both legumes where the *Methylobacterium* induces nodulation and fix nitrogen in the nodules (14, 15). We have identified an efficient nitrogen-fixing leaf-colonizer, strain L2-4 belonging to *Methylobacterium*, from surface-sterilized leaf tissues of *Jatropha curcas*. It is capable of fixing nitrogen to about 634.5 nmol C₂H₄ released h⁻¹ bottle⁻¹ as measured by Hardy's acetylene method (16, 17).

Genome sequencing was carried out using the GS FLX Titanium platform at Macrogen, Inc. (Republic of Korea). The sequence reads were assembled using the GS De Novo Assembler (v2.6). The genome was annotated using the Rapid Annotations using Subsystems Technology server employing the GLIMMER gene caller (18). The draft genome sequence was also annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The draft genome sequence of strain L2-4 included 342 contigs (>500 bp in size), with a calculated genome size 6,800,472 bp long, containing 6,382 protein-coding genes and an average G+C content of 70.8%. A total of 6,255 genes were assigned through the PGAAP and categorized into 6,092 coding sequences, 103 pseudogenes, 5 rRNAs (16S, 23S), 54 tRNAs, 1 noncoding RNA (ncRNA), and 87 frameshifted genes. Comparison of its 16S rRNA genes with EzGenome (<http://ezgenome.ezbiocloud.net/>) using BLASTn revealed that it shares the highest nucleic acid identity with the UV-resistant *Methylobacterium radiotolerans* JCM 2831

(99%), followed by *Methylobacterium* sp. GXF4 (98%) and *Methylobacterium extorquens* AM1 (95%).

Strain L2-4 possesses a conserved cluster of genes associated with photosynthesis, including genes encoding the light-harvesting complex and the reaction center, and genes involved in biosynthesis of bacteriochlorophyll (*bch*) and carotenoids (*crt*). Further analyses of this genome will include comparisons with other *Methylobacterium* genomes already reported (13, 17, 19–21). The genome of strain L2-4 presents several genes involved in metabolic pathways that may contribute to the promotion of plant growth, including genes for the production of auxin biosynthesis, zeatin (*miaA*), cobalamin synthesis protein (*cob*), urea metabolism (*ureABCDEF*), biosorption of heavy metals or decrease of metal toxicity, endoglucanase (*celC*), phytase, C-P lyase system (*phn*), pyrroloquinoline quinone biosynthesis protein (*pqqABCDE*), and methylotrophy gene clusters (*mxa*). In addition, the gene coding for the 1-aminocyclopropane-1-carboxylate deaminase (*acdS*) gene is also observed, which may suggest contributions to plant development under stress conditions (22). The genome information presented here will allow in-depth functional and comparative genome analyses to provide a better understanding of beneficial plant-bacterial associations.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AVNX00000000](http://www.ncbi.nlm.nih.gov/nuccore/AVNX00000000). The version described in this paper is version AVNX01000000.

ACKNOWLEDGMENTS

We thank the Temasek Trust and the Singapore Economic Development Board for the financial funding for the work.

REFERENCES

1. Madhaiyan M, Peng N, Te NS, Hsin I C, Lin C, Lin F, Reddy C, Yan H, Ji L. 2013. Improvement of plant growth and seed yield in *Jatropha curcas* by a novel nitrogen-fixing root associated *Enterobacter* species. *Biotechnol. Biofuels* 6:140. <http://dx.doi.org/10.1186/1754-6834-6-140>.
2. Madhaiyan M, Peng N, Ji L. 2013. Complete genome sequence of *Enterobacter* sp. strain R4-368, an endophytic N-fixing gammaproteobacterium iso-

- lated from surface-sterilized roots of *Jatropha curcas* L. *Genome Announc.* 1(4):e00544-13. <http://dx.doi.org/10.1128/genomeA.00544-13>.
3. Madhaiyan M, Jin TY, Roy JJ, Kim S-J, Weon H-Y, Kwon S-W, Ji L. 2013. *Pleomorphomonas diazotrophica* sp. nov., an endophytic N-fixing bacterium isolated from root tissue of *Jatropha curcas* L. *Int. J. Syst. Evol. Microbiol.* 63:2477–2483. <http://dx.doi.org/10.1099/ij.s.0.044461-0>.
 4. Corpe W, Rheem S. 1989. Ecology of the methylotrophic bacteria on living leaf surfaces. *FEMS Microbiol. Lett.* 62:243–249. <http://dx.doi.org/10.1111/j.1574-6968.1989.tb03698.x>.
 5. Andrews JH, Hirano SS. 1991. Microbial ecology of leaves, vol 499, p 579–580. Springer-Verlag, New York, NY.
 6. Sy A, Timmers AC, Knief C, Vorholt JA. 2005. Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. *Appl. Environ. Microbiol.* 71:7245–7252. <http://dx.doi.org/10.1128/AEM.71.11.7245-7252.2005>.
 7. Elbeltagy A, Nishioka K, Suzuki H, Sato T, Sato Y-I, Morisaki H, Mitsui H, Minamisawa K. 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Sci. Plant Nutr.* 46:617–629. <http://dx.doi.org/10.1080/00380768.2000.10409127>.
 8. Pirttilä AM, Laukkonen H, Pospiček H, Myllylä R, Hohtola A. 2000. Detection of intracellular bacteria in the buds of Scotch pine (*Pinus sylvestris* L.) by *in situ* hybridization. *Appl. Environ. Microbiol.* 66: 3073–3077. <http://dx.doi.org/10.1128/AEM.66.7.3073-3077.2000>.
 9. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, von Mering C, Vorholt JA. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 106:16428–16433. <http://dx.doi.org/10.1073/pnas.0905240106>.
 10. Lacava PT, Araújo WL, Marcon J, Maccheroni W, Azevedo JL. 2004. Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. *Lett. Appl. Microbiol.* 39:55–59. <http://dx.doi.org/10.1111/j.1472-765X.2004.01543.x>.
 11. Van Aken B, Yoon JM, Schnoor JL. 2004. Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (*Populus deltoids* × *nigra* DN34). *Appl. Environ. Microbiol.* 70:508–517. <http://dx.doi.org/10.1128/AEM.70.1.508-517.2004>.
 12. Abanda-Nkpwatt D, Müsch M, Tschiersch J, Boettner M, Schwab W. 2006. Molecular interaction between *Methylobacterium extorquens* and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site. *J. Exp. Bot.* 57:4025–4032. <http://dx.doi.org/10.1093/jxb/erl173>.
 13. Kwak M-J, Jeong H, Madhaiyan M, Lee Y, Sa T-M, Oh TK, Kim JF. 2014. Genome information of *Methylobacterium oryzae*, a plant-probiotic methylotroph in the phyllosphere. *PLoS One* 9:e106704. <http://dx.doi.org/10.1371/journal.pone.0106704>.
 14. Jaftha JB, Strijdom BW, Steyn PL. 2002. Characterization of pigmented methylotrophic bacteria which nodulate *Lotononis bainesii*. *Syst. Appl. Microbiol.* 25:440–449. <http://dx.doi.org/10.1078/0723-2020-00124>.
 15. Sy A, Giraud E, Jourand P, Garcia N, Willemans A, de Lajudie P, Prin Y, Neyra M, Gillis M, Boivin-Masson C, Dreyfus B. 2001. Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. *J. Bacteriol.* 183:214–220. <http://dx.doi.org/10.1128/JB.183.1.214-220.2001>.
 16. Jourand P, Giraud E, Béna G, Sy A, Willemans A, Gillis M, Dreyfus B, de Lajudie P. 2004. *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. *Int. J. Syst. Evol. Microbiol.* 54:2269–2273. <http://dx.doi.org/10.1099/ij.s.0.02902-0>.
 17. Marx CJ, Bringel F, Chistoserdova L, Moulin L, Haque MFU, Fleischman DE, Gruffaz C, Jourand P, Knief C, Lee M-C, Muller EEL, Nadalig T, Peyraud R, Roselli S, Russ L, Goodwin LA, Ivanova N, Kyrpides N, Lajus A, Land ML, Médigue C, Mikhailova N, Nolan M, Woyke T, Stolyar S, Vorholt JA, Vuilleumier S. 2012. Complete genome sequences of six strains of the genus *Methylobacterium*. *J. Bacteriol.* 194:4746–4748. <http://dx.doi.org/10.1128/JB.01009-12>.
 18. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsmma 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. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 19. Vuilleumier S, Chistoserdova L, Lee M-C, Bringel F, Lajus A, Zhou Y, Gourion B, Barbe V, Chang J, Cruveiller S, Dossat C, Gillett W, Gruffaz C, Haugen E, Hourcade E, Levy R, Mangenot S, Muller E, Nadalig T, Pagni M, Penny C, Peyraud R, Robinson DG, Roche D, Rouy Z, Saenampechek C, Salvignol G, Vallenet D, Wu Z, Marx CJ, Vorholt JA, Olson MV, Kaul R, Weissenbach J, Médigue C, Lidstrom ME. 2009. *Methylobacterium* genome sequences: a reference blueprint to investigate microbial metabolism of C1 compounds from natural and industrial sources. *PLoS One* 4:e5584. <http://dx.doi.org/10.1371/journal.pone.0005584>.
 20. Gan HM, Chew TH, Hudson AO, Savka MA. 2012. Genome sequence of *Methylobacterium* sp. strain GXF4, a xylem-associated bacterium isolated from *Vitis vinifera* L. grapevine. *J. Bacteriol.* 194:5157–5158. <http://dx.doi.org/10.1128/JB.01201-12>.
 21. Almeida DM, Dini-Andreote F, Neves AAC, Ramos RTJ, Andreote FD, Carneiro AR, de Souza Lima AO, de Sá PHCG, Barbosa MSR, Araújo WL. 2013. Draft genome sequence of *Methylobacterium mesophilicum* strain SR1. 6/6, isolated from *Citrus sinensis*. *Genome Announc.* 1(3): e00356-13. <http://dx.doi.org/10.1128/genomeA.00356-13>.
 22. Madhaiyan M, Poonguzhalai S, Ryu J, Sa T. 2006. Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta* 224:268–278. <http://dx.doi.org/10.1007/s00425-005-0211-y>.