

Draft Genome Sequence of the Naphthalene Degradator *Herbaspirillum* sp. Strain RV1423

Ruy Jauregui,^a Belén Rodelas,^b Robert Geffers,^c Nico Boon,^d Dietmar H. Pieper,^a Ramiro Vilchez-Vargas^d

Microbial Interactions and Processes Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany^a; Departamento de Microbiología, Facultad de Farmacia, Universidad de Granada, Granada, Spain^b; Genome Analytics, Helmholtz Centre for Infection Research, Braunschweig, Germany^c; Laboratory of Microbial Ecology and Technology (LabMET), University of Ghent, Ghent, Belgium^d

***Herbaspirillum* sp. strain RV1423 was isolated from a site contaminated with alkanes and aromatic compounds and harbors the complete pathway for naphthalene degradation. The new features found in RV1423 increase considerably the versatility and the catabolic potential of a genus of bacteria previously considered mainly to be diazotrophic endophytes to plants.**

Received 19 February 2014 Accepted 27 February 2014 Published 20 March 2014

Citation Jauregui R, Rodelas B, Geffers R, Boon N, Pieper DH, Vilchez-Vargas R. 2014. Draft genome sequence of the naphthalene degrader *Herbaspirillum* sp. strain RV1423. *Genome Announc.* 2(2):e00188-14. doi:10.1128/genomeA.00188-14.

Copyright © 2014 Jauregui 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 Ramiro Vilchez-Vargas, Ramiro.VilchezVargas@UGent.be.

Bacteria of the *Herbaspirillum* genus are commonly described as diazotrophic plant endophytes (1, 2), although recent studies show their greater versatility. *Herbaspirillum* spp. have been found in coastal hydrothermal fields (3), ground water (4), drinking water distribution systems (5), and human sources, including wounds, respiratory tract, gastric juice, feces, urine, and samples from otitis, bacteremia, and cystic fibrosis patients (6–8). Moreover, several studies provided insights into the potential role of *Herbaspirillum* spp. in the biodegradation of fluoranthene (9) and 4-chlorophenol (10). Even though the versatility of *Herbaspirillum* spp. becomes more and more evident, to date, all but one genome project (7) have been focused on root-associated endophytic bacteria, while the genomes of *Herbaspirillum* spp. with catabolic potential remain unexplored.

Here, we present the draft genome sequence of *Herbaspirillum* sp. strain RV1423, isolated from the underground water of a hydrocarbon-contaminated environment (11, 12).

The genome was sequenced using the Illumina MiSeq platform, which generated 8,023,213 paired-end reads of 250 bp assembled using Edena (13, 14), producing 131 contigs with a total genome size of 6.21 Mb (59% G+C content; N_{50} , 129 kbp; mean, 47.41 kb) and an average 300-fold coverage. Automatic annotation was performed using the RAST server version 4.0 (15), generating 5,732 features potentially assigned to protein-coding genes (open reading frames [ORFs]).

Although the 16S rRNA gene sequence shows 99% similarity with that of *Herbaspirillum lusitanum* P6-12, they share only 2,760 ORFs with >80% similarity (amino acid sequences), and 1,472 ORFs observed in the genome of strain RV1423 are absent from the genome of strain P6-12.

Strain RV1423 harbors genes encoding all the enzymes necessary for the mineralization of naphthalene via the gentisate pathway. Only four *Herbaspirillum* genomes (those of *H. lusitanum* P6-12 [16], *H. seropedicae* Os34 [17] and Os45 [18], and *H. huttiense* subsp. *putei* IAM 15032 [19]) harbor two gentisate dioxygenase genes, and RV1423 harbors four gentisate dioxygenases.

Comparing the draft genome of strain RV1423 and those of *Polaromonas naphthalenivorans* CJ2 (20) and *Ralstonia* sp. strain U2 (21), we found high similarities in the genomic regions involved in naphthalene degradation, suggesting that strain RV1423 uses the *nag* pathway (20).

Strain RV1423 harbors two independent *nag* operons, one displaying identical genomic organization and similarity to that previously described in strain CJ2. The second *nag* operon is a fusion between the *nag* operons of strains CJ2 and U2. The fusion occurs in the gene encoding a 2-hydroxychromene carboxylate isomerase. The first 12 genes of the operon show >99% similarity to those of strain CJ2, while the 5 distal genes show >99% similarity to those of strain U2.

A comparison between the draft genome of RV1423 and the 10 genomes/draft genomes of the strains *H. seropedicae* SmR1 (22), P6-12 (16), *Herbaspirillum frisingense* GSF30^T (2), *Herbaspirillum* sp. GW103 (23), Os34 (17), Os45 (18), IAM 15032 (19), CF444, YR522 (3), and JC206 (7) showed that all strains harbor genes for ammonium assimilation and cobalt-zinc-cadmium resistance. The genes for denitrification were found only in strains RV1423, GSF30^T, Os34, Os45, and SmR1. Genes for nitrate and nitrite ammonification were found in all strains but JC206. The genomes of strains RV1423, P6-12, and GSF30^T encode carbon monoxide oxidases, and mercury reductases were found only in strains RV1423 and GW103. Strain RV1423 harbors an alkylmercury lyase that is 94% similar to YP001102036 of *Salmonella enterica* subsp. *enterica* serovar Newport SL254 (24).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in the EMBL-EBI European Nucleotide Archive (ENA) under accession no. [CBXX01000001](https://ena.ebi.ac.uk/ena/record/CBXX01000001) to [CBXX010000131](https://ena.ebi.ac.uk/ena/record/CBXX010000131).

ACKNOWLEDGMENTS

This work was funded by MAGICPAH from the European Commission and the Inter-University Attraction Pole (IUAP) “ μ -manager” funded by the Belgian Science Policy (BELSPO) (grant P7/25).

Special thanks to Agnes Waliczek and Tim Lacoere for technical assistance and to Ana M. Cerdeno-Tarraga from the European Nucleotide Archive for her support in the submission process.

REFERENCES

- Reinhold-Hurek B, Hurek T. 1998. Life in grasses: diazotrophic endophytes. *Trends Microbiol.* 6:139–144. [http://dx.doi.org/10.1016/S0966-842X\(98\)01229-3](http://dx.doi.org/10.1016/S0966-842X(98)01229-3).
- Straub D, Rothballer M, Hartmann A, Ludewig U. 2013. The genome of the endophytic bacterium *H. frisingense* GSF30^T identifies diverse strategies in the *Herbaspirillum* genus to interact with plants. *Front. Microbiol.* 4:168. <http://dx.doi.org/10.3389/fmicb.2013.00168>.
- Brown SD, Utturkar SM, Klingeman DM, Johnson CM, Martin SL, Land ML, Lu TY, Schadt CW, Doktycz MJ, Pelletier DA. 2012. Twenty-one genome sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of *Populus deltoides*. *J. Bacteriol.* 194:5991–5993. <http://dx.doi.org/10.1128/JB.01243-12>.
- Ghosh S, Sar P. 2013. Identification and characterization of metabolic properties of bacterial populations recovered from arsenic contaminated ground water of North East India (Assam). *Water Res.* 47:6992–7005. <http://dx.doi.org/10.1016/j.watres.2013.08.044>.
- Chen L, Jia RB, Li L. 2013. Bacterial community of iron tubercles from a drinking water distribution system and its occurrence in stagnant tap water. *Environ. Sci. Process. Impacts* 15:1332–1340. <http://dx.doi.org/10.1039/c3em00171g>.
- Baldani JJ, Pot B, Kirchhof G, Falsen E, Baldani VL, Olivares FL, Hoste B, Kersters K, Hartmann A, Gillis M, Döbereiner J. 1996. Emended description of *Herbaspirillum*; inclusion of [*Pseudomonas*] *rubrisubalbicans*, a milk plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov.; and classification of a group of clinical isolates (EF group 1) as *Herbaspirillum* species 3. *Int. J. Syst. Bacteriol.* 46:802–810. <http://dx.doi.org/10.1099/00207713-46-3-802>.
- Lagier JC, Gimenez G, Robert C, Raoult D, Fournier PE. 2012. Non-contiguous finished genome sequence and description of *Herbaspirillum massiliense* sp. nov. *Stand. Genomic Sci.* 7:200–209. <http://dx.doi.org/10.4056/sigs.3086474>.
- Spilker T, Uluer AZ, Marty FM, Yeh WW, Levison JH, Vandamme P, Lipuma JJ. 2008. Recovery of *Herbaspirillum* species from persons with cystic fibrosis. *J. Clin. Microbiol.* 46:2774–2777. <http://dx.doi.org/10.1128/JCM.00460-08>.
- Xu HX, Wu HY, Qiu YP, Shi XQ, He GH, Zhang JF, Wu JC. 2011. Degradation of fluoranthene by a newly isolated strain of *Herbaspirillum chlorophenolicum* from activated sludge. *Biodegradation* 22:335–345. <http://dx.doi.org/10.1007/s10532-010-9403-7>.
- Im WT, Bae HS, Yokota A, Lee ST. 2004. *Herbaspirillum chlorophenolicum* sp. nov., a 4-chlorophenol-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* 54:851–855. <http://dx.doi.org/10.1099/ijs.0.02812-0>.
- Kabelitz N, Machackova J, Imfeld G, Brennerova M, Pieper DH, Heipieper HJ, Junca H. 2009. Enhancement of the microbial community biomass and diversity during air sparging bioremediation of a soil highly contaminated with kerosene and BTEX. *Appl. Microbiol. Biotechnol.* 82:565–577. <http://dx.doi.org/10.1007/s00253-009-1868-0>.
- Vilchez-Vargas R, Geffers R, Suárez-Diez M, Conte I, Waliczek A, Kaser VS, Kralova M, Junca H, Pieper DH. 2013. Analysis of the microbial gene landscape and transcriptome for aromatic pollutants and alkane degradation using a novel internally calibrated microarray system. *Environ. Microbiol.* 15:1016–1039. <http://dx.doi.org/10.1111/j.1462-2920.2012.02752.x>.
- Hernandez D, François P, Farinelli L, Osterås M, Schrenzel J. 2008. *De novo* bacterial genome sequencing: millions of very short reads assembled on a desktop computer. *Genome Res.* 18:802–809. <http://dx.doi.org/10.1101/gr.072033.107>.
- Hernandez D, Tewhey R, Veyrieras JB, Farinelli L, Østerås M, François P, Schrenzel J. 2014. *De novo* finished 2.8 Mbp *Staphylococcus aureus* genome assembly from 100 bp short and long range paired-end reads. *Bioinformatics* 30:40–49. <http://dx.doi.org/10.1093/bioinformatics/btt590>.
- 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. <http://dx.doi.org/10.1186/1471-2164-9-75>.
- Weiss VA, Faoro H, Tadra-Sfeir MZ, Raittz RT, de Souza EM, Monteiro RA, Cardoso RL, Wassem R, Chubatsu LS, Huergero LF, Muller-Santos M, Steffens MB, Rigo LU, Pedrosa Fde O, Cruz LM. 2012. Draft genome sequence of *Herbaspirillum lusitanum* P6-12, an endophyte isolated from root nodules of *Phaseolus vulgaris*. *J. Bacteriol.* 194:4136–4137. <http://dx.doi.org/10.1128/JB.00657-12>.
- Ye W, Ye S, Liu J, Chang S, Chen M, Zhu B, Guo L, An Q. 2012. Genome sequence of the pathogenic *Herbaspirillum seropedicae* strain Os34, isolated from rice roots. *J. Bacteriol.* 194:6993–6994. <http://dx.doi.org/10.1128/JB.01934-12>.
- Zhu B, Ye S, Chang S, Chen M, Sun L, An Q. 2012. Genome sequence of the pathogenic *Herbaspirillum seropedicae* strain Os45, isolated from rice roots. *J. Bacteriol.* 194:6995–6996. <http://dx.doi.org/10.1128/JB.01935-12>.
- de Souza V, Piro VC, Faoro H, Tadra-Sfeir MZ, Chicora VK, Guizelini D, Weiss V, Vialle RA, Monteiro RA, Steffens MB, Marchaukoski JN, Pedrosa FO, Cruz LM, Chubatsu LS, Raittz RT. 2013. Draft genome sequence of *Herbaspirillum huttiense* subsp. *putei* IAM 15032, a strain isolated from well water. *Genome Announc.* 1(1):e00252-12. <http://dx.doi.org/10.1128/genomeA.00252-12>.
- Jeon CO, Park M, Ro HS, Park W, Madsen EL. 2006. The naphthalene catabolic (*nag*) genes of *Polaromonas naphthalenivorans* CJ2: evolutionary implications for two gene clusters and novel regulatory control. *Appl. Environ. Microbiol.* 72:1086–1095. <http://dx.doi.org/10.1128/AEM.72.2.1086-1095.2006>.
- Zhou NY, Fuenmayor SL, Williams PA. 2001. *Nag* genes of *Ralstonia* (formerly *Pseudomonas*) sp. strain U2 encoding enzymes for gentisate catabolism. *J. Bacteriol.* 183:700–708. <http://dx.doi.org/10.1128/JB.183.2.700-708.2001>.
- Pedrosa FO, Monteiro RA, Wassem R, Cruz LM, Ayub RA, Colauto NB, Fernandez MA, Fungaro MH, Grisard EC, Hungria M, Madeira HM, Nodari RO, Osaku CA, Petzl-Erler ML, Terenzi H, Vieira LG, Steffens MB, Weiss VA, Pereira LF, Almeida MI, Alves LR, Marin A, Araujo LM, Balsanelli E, Baura VA, Chubatsu LS, Faoro H, Favetti A, Friedermann G, Glienke C, Karp S, Kava-Cordeiro V, Raittz RT, Ramos HJ, Ribeiro EM, Rigo LU, Rocha SN, Schwab S, Silva AG, Souza EM, Tadra-Sfeir MZ, Torres RA, Dabul AN, Soares MA, Gasques LS, Gimenes CC, Valle JS, Ciferri RR, Correa LC, Murace NK, et al. 2011. Genome of *Herbaspirillum seropedicae* strain SmR1, a specialized diazotrophic endophyte of tropical grasses. *PLoS Genet.* 7:e1002064. <http://dx.doi.org/10.1371/journal.pgen.1002064>.
- Lee GW, Lee KJ, Chae JC. 2012. Genome sequence of *Herbaspirillum* sp. strain GW103, a plant growth-promoting bacterium. *J. Bacteriol.* 194:4150. <http://dx.doi.org/10.1128/JB.00806-12>.
- Fricke WF, Mammel MK, McDermott PF, Tartera C, White DG, Leclerc JE, Ravel J, Cebula TA. 2011. Comparative genomics of 28 *Salmonella enterica* isolates: evidence for CRISPR-mediated adaptive sub-lineage evolution. *J. Bacteriol.* 193:3556–3568. <http://dx.doi.org/10.1128/JB.00297-11>.