Draft genome of *Paraburkholderia caballeronis* TNe-841T, a free-living, nitrogen-fixing, tomato plant-associated bacterium

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**Abstract**

*Paraburkholderia caballeronis* is a plant-associated bacterium. Strain TNe-841T was isolated from the rhizosphere of tomato (*Solanum lycopersicum* L. var. *lycopersicum*) growing in Nepantla Mexico State. Initially this bacterium was found to effectively nodulate *Phaseolus vulgaris* L. However, from an analysis of the genome of strain TNe-841T and from repeat inoculation experiments, we found that this strain did not nodulate bean and also lacked nodulation genes, suggesting that the genes were lost. The genome consists of 7,115,141 bp with a G + C content of 67.01%. The sequence includes 6251 protein-coding genes and 87 RNA genes.

**Keywords:** Paraburkholderia *caballeronis*, Tomato plant, Rhizosphere, Nitrogen fixation, Root nodulation

**Introduction**

*Paraburkholderia caballeronis* was isolated in the State of Mexico, Mexico from the tomato rhizosphere as a free-living, nitrogen-fixing bacterial species [1]. It was described as *B. caballeronis* and found to nodulate *Phaseolus vulgaris* L. [2]. Most nodulating bacteria are isolated from root nodules but this was not the case for *B. caballeronis*, which was isolated from rhizospheric soil. Given the ability of this bacterium to fix nitrogen under both free-living and symbiotic conditions, this type strain was selected for genome sequencing to study its nitrogen-fixing and other plant-growth promoting activities. However, after analyzing the genome, we found that the genes for fixing nitrogen were present but nodulation genes were not. We carried out several unsuccessful tests to check the ability of this strain to nodulate *P. vulgaris*, strongly suggesting that the strain had lost the *nod* genes.

The genome sequence of *P. caballeronis* TNe-841T was obtained in cooperation with JGI-DOE. The type species is TNe-841T (=LMG 26416T = CIP 110324T).

**Organism information**

**Classification and features**

*Burkholderia caballeronis* TNe-841T has been proposed to belong to the newly described genus *Paraburkholderia*. The last years, *Burkholderia* sensu lato has been subjected to some taxonomical changes, where the genus has been split to *Burkholderia*, *Paraburkholderia*, *Caballeronia* and *Robbsia andropogonis* [3–5]. However, this division has caused some skepticism, which has been expressed by The International Committee on Systematics of Prokaryotes, through the Subcommittee for the Taxonomy of *Rhizobium* and *Agrobacterium* discussed during the 12th Nitrogen Fixation Conference held in Budapest, Hungary on 25 August 2016 [6]. The Subcommittee stated: “Research efforts directed towards robust characterization and taxonomy of *Burkholderia* sensu lato species can help in realizing this agricultural...
Fig. 1 Phylogenetic tree highlighting the position of *Paraburkholderia caballeronis* TNE-841\(^\text{T}\) in relation to other *Paraburkholderia* species. *Burkholderia* and *Robbsia* were used as outgroups. The bar represents the number of expected substitutions per site under the GTR + G model. The sequenced strain is indicated in red.
potential. Clearly, large-scale phylogenomic study is required for resolving these taxa. In order to analyze this issue and to provide generic limits in *Burkholderia* sensu lato, a large phylogenomic analysis was carried out using the amino acid and nucleotide sequence of 106 conserved proteins from 92 species [7]. The analysis performed with maximum likelihood unambiguously supported five different lineages: *Burkholderia* sensu stricto, *Paraburkholderia*, *Caballeronia*, *Robbsia andropogonis* and *B. rhizoxiniaca*. To check the position of *P. caballeronis* within *Paraburkholderia*, the 16S rRNA gene sequence (ca. 1500 bp) was amplified and sequenced at Macrogen [8] with the universal primers fD1/rD1 [9]. The nucleotide sequence (accession number EF139186) was compared to other *Paraburkholderia* species using Muscle 3.57 for alignment [10]. A phylogenetic analysis was performed with ML using the PhyML program [11]. Among-site rate variation was modeled by a gamma distribution with four rate categories [12] with each category being represented by its mean under the GTR + G model. Tree searches were initiated from a BioNJ seed tree retaining the best tree among those found with NNI (Nearest Neighbor Interchange). The robustness of the ML topologies was evaluated using a Shimodaira-Hasegawa (SH)-like test [13]. The ML tree was obtained with the program MEGA version 5 [14]. The position of *P. caballeronis* in the ML tree shows that it is close to *P. kururiensis* (Fig. 1). The colony morphology on BSE medium was uniform, 1 mm diameter, with entire margins that were convex, whitish, and translucent. The cells are strictly aerobic Gram-negative, non-spore forming rod (0.49–0.69 μm x 1.2–2.7 μm) and have flagella (Fig. 2). Other phenotypic traits for this strain have been published before [2]. The strain has the following enzymes: arginine dihydro-lase, urease catalase, and nitrogenase and associated proteins. It is also able to assimilate D-glucose, DL-arabinose, D-mannose, D-mannitol, N-acetyl glucosamine, gluconate, capric acid, malate acetate, D-ribose, D-xylene, D-adonitol, D-galactose, D-fuctose, L-rhamnose, inositol, D-sorbitol, D-cellobiose, D-turanose, D-xylene, D-fucose, D-arabitol, potassium 2-ketogluconate, and potassium 5-ketogluconate (Table 1). Oxidase activity was weak. The strain grew on MacConkey agar plates at 29 °C and 37 °C, but weakly at 42 °C. *P. caballeronis* TNe-841T grew on LB and BSE agar plates at 15, 29, 37, and 42 °C and on LB plates at 29 °C with up to 5.0% NaCl.

**Chemotaxonomic data**

The following fatty acids were detected in strain TNe-841T [2]: C14:0 (4.46%), C16:0 (21.77%), C16:0 2OH (2.3%), C16:0 3OH (6.2%), C16:1 2OH (3.81%), C17:0 cyclo (12.43%), C18:1 2OH (1.5%), C18:1 ω 7c (16.62%), C19:0 cyclo ω 8c (14.89%), summed feature 2 (5.9%), and summed feature 3 (8.3%). Summed feature two corresponds to C14:0 3OH and/or 16:1 ISO I, an unidentified fatty acid with equivalent chain length value of 10.928 12:0 ALDE or any combination of these fatty acids. Summed feature three corresponds to C16:1 w7c and/or C15:0 ISO 2OH.

**Genome sequencing information**

**Genome project history**

*P. caballeronis* TNe-841T was sequenced at the JGI-DOE as a part of the project “Root nodule microbial
communities of legume samples collected from USA, Mexico and Botswana directed by Dr. Ann M. Hirsch. The goal of this project was to identify the microbial community housed within nodules of native legumes living in three arid or semi-arid, nutrient-poor environments in Mexico, Botswana, and the United States. Both Paraburkholderia and Rhizobium bacteria had been previously isolated from Mexico. \textit{P. caballeronis} TNe-841\textsuperscript{T} was chosen as the reference strain for a study of bacteria associated with native legume soils and nodules.

The complete sequence was finished on May 2015 and some features are presented in Table 2 and Fig. 3.

### Table 1 Classification and general features of \textit{Paraburkholderia caballeronis} strain TNe-841\textsuperscript{T} \cite{26}

| MIGS ID | Property | Term | Evidence code$^a$ |
|---------|----------|------|-------------------|
|        | Classification | Domain Bacteria | TAS [27] |
|        | Phylum | Proteobacteria | TAS [28] |
|        | Class | Betaproteobacteria | TAS [29] |
|        | Order | Burkholderiales | TAS [30] |
|        | Family | Burkholderiaceae | TAS [31] |
|        | Genus | Paraburkholderia | TAS [32] |
|        | Species | \textit{Paraburkholderia caballeronis} | TAS [2] |
|        | Type strain | TNe-841\textsuperscript{T} (LMG 26416 = CIP 110324) | TAS [2] |
|        | Gram stain | Negative | TAS [2] |
|        | Cell shape | Cells are single coccoids or in pairs | TAS [2] |
|        | Motility | Motile | TAS [2] |
|        | Sporulation | Non-spore forming | TAS [2] |
|        | Temperature range | 15-42 °C | TAS [2] |
|        | Optimum temperature | 30 °C | TAS [2] |
|        | pH range; Optimum | 6-7; 6 | IDA |
|        | Carbon source | D-glucose, DL-arabinose, D-mannose, D-mannitol, N-acetyl glucosamine, gluconate, capric acid, malate, acetate, D-ribose, D-xylose, D-adonitol, D-galactose, D-fructose, L-rhamnos, inositol, D-sorbitol, D-cellubiose, D-turanose, D-xylose, D-fucose, D-aritbol, potassium 2-ketogluconate, and potassium 5-ketogluconate | TAS [2] |

- Habitat: Tomato rhizosphere soil
- Salinity: Up to 5.0% NaCl (w/v)
- Oxygen requirement: Aerobic
- Pathogenicity: Non-pathogen
- Geographic location: Mexico/Estado de México
- Sample collection: 2006
- Latitude: 18°59′11.7″ N (18.986589)
- Longitude: 98°50′44.0″ W (−98.845552)
- Altitude: 2010 m

$^a$ Evidence codes - IDA Inferred from Direct Assay, TAS Traceable Author Statement (i.e. a direct report exists in the literature), NAS Non-traceable Author Statement (i.e. not directly observed for the living isolated sample but based on a generally accepted property for the species or anecdotal evidence). These evidence codes are from the Gene Ontology project \cite{33}.

Growth conditions and genomic DNA preparation
\textit{P. caballeronis} TNe-841\textsuperscript{T} cells were grown in 5 ml of LB minus NaCl at 30 °C for 18 h at 120 rpm. The DNA extraction was done using Invitrogen’s Purelink™ Genomic DNA Mini Kit. The purified DNA was monitored for integrity by gel electrophoresis, and then sent to the JGI for sequencing.

Two surface-sterilized and rinsed seeds of \textit{Phaseolus vulgaris} L. c.v. Negro Chapingo were planted per pot in surface-sterilized black pots (29.5 cm tall; 17 cm diameter) filled with autoclaved vermiculite:perlite (2:1) and watered with autoclaved 1/4 strength Hoagland’s –N medium. Two separate experiments were performed. The
pots were either left uninoculated (sterilized water or Hoagland’s –N medium was added), inoculated with 10 ml of *P. caballeronis* TNe-841T diluted to OD 600 = 0.2 or with *B. tuberum* DUS833, which was a positive control. Some pots were also watered with 1/4 strength Hoagland’s + N medium as an additional positive control. The appropriate medium was added twice weekly and the plants grown in a Conviron growth chamber under 16 h days/8 h nights at 24 °C.

**Genome sequencing and assembly**

The draft genome of *P. caballeronis* was generated using the PacBio sequencing technology [15]. A Pacbio SMRTbell™ library was constructed and sequenced on the PacBio RS platform, which generated 194,884 filtered sub-reads totaling 879.3 Mbp. All general aspects of library construction and sequencing performed at the JGI can be found at [16]. The raw reads were assembled using HGAP (version: 2.3.0 p5 protocol version = 2.3.0 method = RS HGAP Assembly.3 smrtpipe.py v1.87.139483) [17]. The final draft assembly contained 3 contigs in 3 scaffolds totaling 7.115 Mbp in size. The input read coverage was 62.2X.

**Genome annotation**

Genes were identified using Prodigal [18] followed by a round of manual curation using GenePRIMP [19] for finished genomes and draft genomes in fewer than

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**Table 2** Project information

| MIGS ID | Property                     | Term                                |
|---------|------------------------------|-------------------------------------|
| MIGS 31 | Finishing quality            | Level 3: Improved-High-Quality-Draft|
| MIGS-28 | Libraries used               | PacBio SMRTbell™                    |
| MIGS 29 | Sequencing platforms         | PacBio RS PacBio RS II              |
| MIGS 31.2| Fold coverage               | 62.2X                               |
| MIGS 30 | Assemblers                   | HGAP version 2.3.0_p5               |
| MIGS 32 | Gene calling method          | Prodigal                            |
| GenBank ID |                            | PRJEB16390                          |
| GenBank Date of Release | | October 20th 2016              |
| GOLD ID |                            | Gp115207                            |
| BIOPROJECT |                          | PRJNA332775                         |
| MIGS 13 | Source Material Identifier   | LMG 26416T = CIP 110324T            |
| Project relevance |                | Environmental                       |

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**Fig. 3** Graphical map of the 3 scaffolds of the genome of *Paraburkholderia caballeronis* TNe-841T. From the bottom to the top of each scaffold:

- Genes on forward strand (color by COG categories as denoted by the IMG platform).
- Genes on reverse strand (color by COG categories).
- RNA genes (tRNAs green, sRNAs red, other black).
- GC content, GC Skew.
10 scaffolds. The predicted CDSs were translated and used to search the NCBI nonredundant database, UniProt, TIGRFam, Pfam, KEGG, COG, and InterPro databases. The tRNAscanSE tool [20] was used to find tRNA genes whereas ribosomal RNA genes were found by searches against models of the ribosomal RNA genes built from SILVA [19]. Other non-coding RNAs such as the RNA components of the protein secretion complex and the RNase P were identified by searching the genome for the corresponding Rfam profiles using INFERNAL [20]. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes platform [21] developed by the JGI Walnut Creek CA USA [21].

The genome was also manually annotated at IPN and UCLA using the IMG platform [21].

**Genome properties**

The final draft assembly of *P. caballeronis* TNe-841T contained 3 contigs in 3 scaffolds accumulating 7,115,141 bp in size (Table 3). The G + C content of the genome was 67.01%, which is very close to the one determined during the description of the species (66.0%) [2]. The genome was predicted to encode 6338 genes including 6251 protein-coding genes and 87 RNA genes (15 rRNAs, 60 tRNAs and 12 ncRNA). The number of genes associated with general COG functional categories is shown in Table 4, in addition to other functions such as extracellular structures and mobilome.

**Table 3** Genome statistics

| Attribute                  | Value            | % of Total |
|----------------------------|------------------|------------|
| Genomic size (bp)          | 7,115,141        | 100.00     |
| DNA coding (bp)            | 6,194,680        | 87.06      |
| DNA G+C (bp)               | 4,767,529        | 67.01      |
| DNA scaffolds              | 3                | 100.00     |
| Total genes                | 6338             | 100.00     |
| Protein coding genes       | 6251             | 98.63      |
| RNA genes                  | 87               | 98.63      |
| Pseudo genes               | 123              | 1.94       |
| Genes in internal clusters | 515              | 8.13       |
| Genes with function prediction | 5088             | 80.28      |
| Genes assigned to COGs     | 4633             | 73.10      |
| Genes with Pfam domains    | 5352             | 84.44      |
| Genes with signal peptides | 585              | 9.23       |
| Genes with transmembrane helices | 1456             | 22.97      |
| CRISPR repeats             | NF               |            |

**Table 4** Number of genes associated with general COG functional categories

| Code | Value | %age | Description                                      |
|------|-------|------|-------------------------------------------------|
| J    | 226   | 4.25 | Translation ribosomal structure and biogenesis  |
| A    | 1     | 0.02 | RNA processing and modification                 |
| K    | 492   | 9.25 | Transcription                                   |
| L    | 124   | 2.33 | Replication recombination and repair            |
| B    | 1     | 0.02 | Chromatin structure and dynamics                |
| D    | 34    | 0.64 | Cell cycle control Cell division chromosome partitioning |
| V    | 98    | 1.84 | Defense mechanisms                              |
| T    | 274   | 5.15 | Signal transduction mechanisms                  |
| M    | 361   | 6.79 | Cell wall/membrane biogenesis                   |
| N    | 132   | 2.48 | Cell motility                                   |
| U    | 116   | 2.18 | Intracellular trafficking and secretion         |
| O    | 180   | 3.39 | Posttranslational modification protein turnover chaperones |
| C    | 376   | 7.07 | Energy production and conversion                |
| G    | 367   | 6.9  | Carbohydrate transport and metabolism           |
| E    | 520   | 9.78 | Amino acid transport and metabolism             |
| F    | 102   | 1.92 | Nucleotide transport and metabolism             |
| H    | 285   | 5.36 | Coenzyme transport and metabolism               |
| I    | 300   | 5.64 | Lipid transport and metabolism                  |
| P    | 338   | 6.36 | Inorganic ion transport and metabolism          |
| Q    | 190   | 3.57 | Secondary metabolites biosynthesis transport and catabolism |
| R    | 514   | 9.67 | General function prediction only                |
| S    | 213   | 4.01 | Function unknown                                |
| 1705 |       | 26.9 | Not in COGs                                    |

The total is based on the total number of protein coding genes in the genome.

**Insights from the genome sequence**

*P. caballeronis* was originally described as a free-living, nitrogen-fixing bacteria with the ability to form nodules on *Phaseolus vulgaris* L. roots [2]. Although nitrogen fixation genes are present, nodulation genes were not found in the sequenced genome. Moreover, after the initial experiments, *P. vulgaris* nodulation was no longer detected in greenhouse bioassays in two different laboratories. This nodulation instability seems to be more frequent than originally assumed because a similar loss of nodulation ability has been reported with other *Burkholderia* strains isolated from nodules. The strains CCGE1002 and CCGE1003 (Marco Antonio Rogel CCG-UNAM, pers. comm.) also lost the ability to nodulate, but strain CCGE1002, which retains the ability to nodulate, was recovered from a stored sample. Its symbiotic plasmid was subsequently sequenced (NCBI BioSample PRJNA37719). In contrast, nodulation genes were no longer detected in
the genome of strain CCGE1003 (NCBI BioSample PRJNA37721). A similar loss of nodulation genes was reported for two Burkholderia strains isolated from Kennedia coccinea [22] and Gastrolobium capitatum [23] in Australia.

Strain TNe-841\(^T\) also contains genes for degrading a large number of xenobiotics including aminobenzoate, atrazine, benzoate, bisphenol, caprolactam, chloroalkane, chloroalkene, chlorohexane, chlorobenzene, dioxin, ethylbenzene, fluorobenzoate, naphthalene, nitrotoluene, polycyclic aromatic hydrocarbons, styrene, toluene, and xylene.

ANI calculation was used to compare the genome of \(P. \) caballeronis TNe-841\(^T\) and other Paraburkholderia species (Table 5). The ANI results showed that strains TNe-851\(^T\) correspond to a different species since the highest ANI value was 83.32. The accepted ANI cut-off for species is 95-96%, which corresponds to a DNA-DNA hybridization of 70% [24, 25].

### Conclusions

\(P. \) caballeronis TNe-81\(^T\), is a plant-associated bacteria species with the ability to fix nitrogen, although the ability to nodulate legumes as shown in the original description was apparently lost. This nodulation instability seems to be rather common among nodulating bacteria, particularly Burkholderia/Paraburkholderia. Our interest in studying the genome of \(P. \) caballeronis TNe-841\(^T\) started when we found that this bacterium, isolated from the tomato rhizosphere, was able to nodulate bean. This led us to find out the identity of the original host for this species. Our work team has recently isolated a \(P. \) caballeronis strain from bean nodules used as a trap with soil from an area where Mimosoideae plants are present (unpublished results). We are characterizing additional isolates from Mimosoideae plant nodules to try to establish if this plant might be the host of \(P. \) caballeronis TNe-841\(^T\).

### Table 5 Average nucleotide identity of strain TNe-841\(^T\) with Paraburkholderia species genome

| Paraburkholderia species         | Average Nucleotide Identity (%) |
|----------------------------------|---------------------------------|
| P. acidipaludis NBRC 101816 \(^T\) | 83.32                           |
| P. ferrariae NBRC 106233 \(^T\)  | 83.22                           |
| P. tropica LMG 22274 \(^T\)      | 83.05                           |
| P. unamare MTI-641 \(^T\)       | 82.96                           |
| P. mimosarum LMG 23256 \(^T\)   | 82.77                           |
| P. silvatlantica SRWh-20 \(^T\)  | 82.77                           |
| P. heleia NBRC 101817 \(^T\)    | 82.68                           |
| P. nodosa DSM 21604 \(^T\)      | 82.68                           |
| P. oxyphila NBRC 105797 \(^T\)  | 82.64                           |
| P. sacchari LMG 19450 \(^T\)    | 82.59                           |
| P. mimosarum STM3621 \(^T\)     | 82.58                           |
| P. eburnea LMG 29537 \(^T\)     | 82.36                           |
| P. bannensis NBRC 103871 \(^T\) | 82.31                           |
| P. kuriensis JCM 10599 \(^T\)   | 81.96                           |
| P. santisol LMG 24000 \(^T\)    | 81.82                           |
| P. susongensis LMG 29450 \(^T\) | 81.82                           |
| P. tuberum STM678 \(^T\)        | 81.62                           |
| Robbsia andropogonis Ba3549     | 73.75                           |

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### Authors’ contributions

FURR prepared bacteria for shipping to UCLA Laboratory and analysed data. EYTG performed bean inoculation at IPN. MM and EH prepared the DNA for sequencing and did the bean inoculations at UCLA. MH, AC, MP, KP, NV, NM, DS, TBKR, VM, NI, NK, TW and NS performed the technical work for sequencing, assembly, and annotation of the genome. AMH led the manual annotation group at UCLA and wrote and reviewed the final manuscript. PES analysed data and drafted the manuscript. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

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