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Paraburkholderia aromaticivorans sp. nov., an aromatic hydrocarbon-degrading bacterium, isolated from gasoline-contaminated soil

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Abstract

A Gram-stain-negative, facultatively aerobic, aromatic hydrocarbon-degrading bacterium, designated strain BN5^T, was isolated from gasoline-contaminated soil. Cells were motile and slightly curved rods with a single flagellum showing catalase and oxidase activities. Growth was observed at 20–37 °C (optimum, 25–30 °C), pH 3–7 (optimum, pH 5–6) and 0–2 % NaCl (optimum, 0 %). Ubiquinone-8 was the predominant respiratory quinone. The major fatty acids were $C_{16:0}$, cyclo-$C_{19:0}\omega8c$ and summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified phosphoamino lipid, three unidentified amino lipids and eight unidentified lipids were the identified polar lipids. The DNA G+C content was 62.93 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain BN5^T formed a phylogenic lineage with members of the genus *Paraburkholderia* and showed the highest 16S rRNA gene sequence similarities to *Paraburkholderia phytofirmans* PsJN^T (99.4 %), *Paraburkholderia dipogonis* DL7^T (98.8 %) and *Paraburkholderia insulsa* PNG-April^T (98.8 %). The average nucleotide identity and *in silico* DNA–DNA hybridization (DDH) values between strain BN5^T and *P. phytofirmans* PsJN^T were 88.5 and 36.5 %, respectively. The DDH values for strain BN5^T with *P. dipogonis* LMG 28415^T and *P. insulsa* DSM 28142^T were 41.0±4.9 % (reciprocal, 33.0±4.3 %) and 47.1±6.6 % (reciprocal, 51.7±5.4 %), respectively. Based on its physiological, chemotaxonomic and phylogenetic features, we conclude that strain BN5^T is a novel species of the genus *Paraburkholderia*, for which the name *Paraburkholderia aromaticivorans* sp. nov. is proposed. The type strain is BN5^T (=KACC 19419^T=JCM 32303^T).

The genus *Burkholderia* was first proposed in 1992 by Yabuuchi *et al.* [1], with *Burkholderia cepacia* as the type species, to accommodate seven species of the genus *Pseudomonas* homology group II [2]. Since then, more than 100 species of *Burkholderia* new have been described. However, the genus *Burkholderia* was also not monophyletic; it eventually split based on 16S rRNA gene sequences and conserved sequence indels (CSIs), and the genus *Paraburkholderia* was established, with *Paraburkholderia graminis* as the type species [3]. At the time of writing, the genus *Paraburkholderia* includes 65 species with validly published names (www.bacterio.net/paraburkholderia.html). Although the genus *Burkholderia* includes some animal and plant pathogens, no pathogenic strains have been reported in the genus *Paraburkholderia* [3, 4]. Cells of the genus *Paraburkholderia* are Gram-stain-negative, straight, slightly curved, or sometimes coccoid rods, with one or more polar flagella. Cell size varies, ranging from 0.4 to 1.2 µm wide and 1.2–3.0 µm long and the DNA G+C contents are 58.9–65.0 mol%. Members of the genus *Paraburkholderia* have been isolated from diverse ecological niches such as soil [5–11], rhizosphere [10–12], plants [13–16], arsenic-rich marine sediment [17], polluted soil [18–20] and a weathered rock surface [21]. In this study, we isolated a putative novel strain belonging to the genus *Paraburkholderia*, designated strain BN5^T, from gasoline-contaminated soil and characterized it taxonomically using a polyphasic approach.

A novel aromatic hydrocarbon-degrading bacterium, strain BN5^T, was isolated from a gasoline-contaminated soil sample obtained near a gas station located in Yangju, Kyeonggi province, Republic of Korea (37° 50′ 21.5″ N 126° 59′ 37.2″ E). To enrich aromatic hydrocarbon-degrading bacteria, a soil extract broth was prepared by resuspending 800 g gasoline-contaminated soil in 2 l distilled water. The supernatant

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Abbreviations: ANI, average nucleotide identity; BTEX, benzene, toluene, ethylbenzene and *o*-, *m*-, *p*-xylene; CSIs, conserved sequence indels; DDH, DNA-DNA hybridization; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; Q-8, ubiquinone-8.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and the genome sequences of strain BN5^T are MF817715 and NZ_CP022989.1–96.1, respectively.
One supplementary table and three supplementary figures are available with the online version of this article.

from the resuspended soil mixture was centrifuged (3745 ***g***, 10 min) and filtered using a 0.45 µm membrane filter (Millipore). Approximately 10 g gasoline-contaminated soil sample was added to a cotton-plugged 500 ml Erlenmeyer flask containing 100 ml soil extract broth and 1 ml benzene, toluene, ethylbenzene and *o*-, *m*-, *p*-xylene mixture (BTEX; 1 : 1 : 1 : 1 : 1 : 1). The enrichment culture was incubated with shaking (180 r.p.m.) at 25 °C and then subcultured into 100 ml fresh soil extract broth containing 1 ml BTEX mixture every 2 weeks, for a total of three times. The final enrichment culture was serially diluted in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2) and spread onto Reasoner's 2A agar (R2A; BD) and incubated aerobically at 25 °C until colonies appeared. The 16S rRNA genes of colonies grown on R2A agar were PCR amplified using the F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) primers, and double digested with *Hae*III and *Hha*I [22, 23]. The restriction fragment pattern was used to classify the colonies, and all PCR products showing different restriction fragment patterns were partially sequenced using the 340F primer (5′-CCT ACG GGA GGC AGC AG-3′). The obtained 16S rRNA gene sequences were compared to those of type strains using the Nucleotide Similarity Search program of the EzTaxon-e server (www.ezbiocloud.net/) [24]. A presumably novel strain belonging to the genus *Paraburkholderia*, designated BN5^T, was selected for further phenotypic and phylogenetic analyses. Strain BN5^T was routinely cultured on R2A agar at 25 °C for 3 days, except where indicated, and stored at −80 °C in R2A broth supplemented with 15 % (v/v) glycerol for long-term preservation. The type strains of *Paraburkholderia phytofirmans* (KACC 15011^T), *Paraburkholderia dipogonis* (LMG 28415^T), *Paraburkholderia insulsa* (DSM 28142^T) and *Paraburkholderia graminis* (DSM 17151^T) were obtained for use as reference strains.

The 16S rRNA gene of strain BN5^T was PCR amplified using primers F1 and R13 and then ligated into the pCR2.1 vector using the TOPO cloning kit (Invitrogen) according to the manufacturer's instructions. The resulting construct was sequenced using the M13 reverse and T7 primers in the TOPO cloning kit by Macrogen (Republic of Korea) to obtain an almost-complete 16S rRNA gene sequence (1455 nucleotides). Identification of phylogenetic neighbours for tree reconstructions and calculation of pairwise 16S rRNA gene sequence similarities were performed by using the EzTaxon-e database [24]. The 16S rRNA gene sequences of strain BN5^T and closely related type strains were aligned using the fast secondary-structure aware Infernal aligner of the Ribosomal Database Project (https://pyro.cme.msu.edu/aligner/form.spr) [25]. The phylogenetic relationships between strain BN5^T and closely related type strains were inferred using the DNADIST and DNAPARS programs based on the neighbour-joining (NJ) algorithm with Kimura's two-parameter model and the maximum-parsimony (MP) algorithm through a heuristic search, respectively, in the PHYLIP software package (version 3.695) [26], and their tree topologies were evaluated through bootstrap analysis based on 1000 resamplings. The maximum-likelihood (ML) analysis with bootstrap values was performed using RAxML-HPC BlackBox (version 8.2.9) in the Cyber-Infrastructure for Phylogenetic Research project (www.phylo.org) [27]. The whole genome of strain BN5^T was extracted using the Wizard Genomic DNA Purification Kit (Promega) and sequenced on an Illumina HiSeq 2500 platform at Macrogen (Republic of Korea). Conserved sequence indels (CSIs) among strain BN5^T and closely related *Paraburkholderia* and *Burkholderia* species with publicly available genome information were analysed using CLUSTAL Omega (www.ebi.ac.uk/Tools/msa/clustalo/), as described previously [3]. The average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (DDH) values between strain BN5^T and closely related *Paraburkholderia* species were calculated using a stand-alone program available in the EZGenome web server (www.ezbiocloud.net/sw/oat) [28] and the server-based Genome-to-Genome Distance Calculator version 2.1 (http://ggdc.dsmz.de/distcalc2.php) [29], respectively. DNA–DNA relatedness between strain BN5^T and reference strains, *P. dipogonis* (LMG 28415^T) and *P. insulsa* (DSM 28142^T), was evaluated by DDH using a genome-probing microarray method, as described by Chang *et al.* [30]. The DDH experiments were confirmed by reciprocally interchanging the probes and target DNA.

Phylogenetic analysis based on the 16S rRNA gene sequences using the ML algorithm indicated that strain BN5^T clearly formed a distinct phylogenetic lineage within the genus *Paraburkholderia* (Fig. 1), which was also supported by phylogenetic analyses using the NJ and MP algorithms (data not shown). Analysis of 16S rRNA gene sequence similarities revealed that strain BN5^T had the highest sequence similarities to *P. phytofirmans* PsJN^T (99.4 %), *P. dipogonis* DL7^T (98.8 %), *P. insulsa* PNG-April^T (98.8 %), *Paraburkholderia fungorum* NBRC 102489^T (98.6 %), *Paraburkholderia kirstenboschensis* Kb15^T (98.5 %), *Paraburkholderia caledonica* NBRC 102488^T (98.5 %), *Paraburkholderia xenovorans* LB400^T (98.4 %) and *Paraburkholderia terricola* LMG 20594^T (98.3 %). The genome of strain BN5^T consisted of two circular chromosomes of 4.38 Mb (NZ_CP022989.1) and 2.99 Mb (NZ_CP022990.1), and six circular plasmids (0.66, 0.49, 0.16, 0.15, 0.04 and 0.04 Mb). The entire genome (8.91 Mb) contained 7756 predicted protein-coding sequences. CSI analysis showed that strain BN5^T shared CSIs with *Paraburkholderia* species, but not with *Burkholderia* species, in four highly conserved proteins (transposase A-like protein, group 1 glycosyl transferase, 4-hydroxyacetophenone monooxygenase and undecaprenyl-phosphate glucose phosphotransferase; Fig. S1, available in the online version of this article), which suggested that strain BN5^T belongs to the genus *Paraburkholderia*. The ANI and *in silico* DDH values were calculated between strain BN5^T and closely related type strains of the genus *Paraburkholderia* for which genome information is publicly available. The ANI and *in silico* DDH values for strain BN5^T and the type strains of *P. phytofirmans*, *P. fungorum*, *P. kirstenboschensis*,

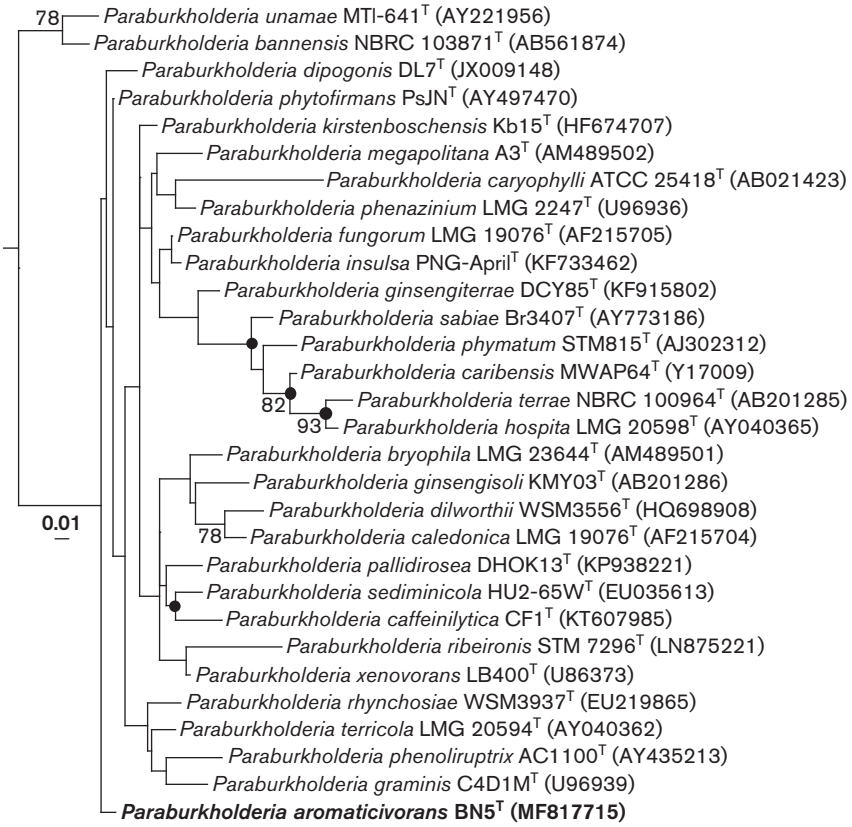

Fig. 1. A maximum-likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationships among strain BN5^T and related taxa. Bootstrap values greater than 70 % are shown on the nodes as percentages of 1000 replicates. Filled circles (•) indicate that the corresponding nodes were also recovered in the neighbour-joining and maximum-parsimony trees. *Cupriavidus necator* ATCC 43291^T (AF191737) was used as an outgroup (not shown). Bar, 0.01 changes per nucleotide position.

P. caledonica, *P. xenovorans* and *P. terricola* were 88.5 and 36.5 %, 84.9 and 29.9 %, 83.6 and 28.8 %, 83.2 and 27.9, 90.5 and 41.6 % and 86.0 % and 31.7 %, respectively, which were clearly lower than the thresholds (95 and 70 %, respectively) generally accepted for species delineation [31, 32].

Because it has been suggested that 98.65–98.7 % 16S rRNA gene sequence similarity between two strains equates to 70 % DNA–DNA relatedness, the gold standard for species delineation [31–34], DDH experiments were performed between strain BN5^T and *Paraburkholderia* species with more than 98.65–98.7 % of 16S rRNA gene sequence similarities, without publicly available genome information. The DDH relatedness values between strain BN5^T and the type strains of *P. dipogonis* (LMG 28415^T) and *P. insulsa* (DSM 28142^T) were 41.0±4.9 % (reciprocal, 33.0 ±4.3 %) and 47.1±6.6 % (reciprocal, 51.7±5.4 %), respectively. These values were clearly lower than the criterion (70 %) for species delineation [31, 33, 34]. Analysis of the CSIs and the ANI and DDH values clearly suggested that strain BN5^T represents a novel species of the genus *Paraburkholderia*.

The naphthalene and BTEX biodegrading abilities of strain BN5^T were tested in serum bottles containing naphthalene or a BTEX mixture in minimal salt basal medium [35], as described previously [36, 37]. Growth of strain BN5^T was assessed at 25 °C for 3 days on several bacteriological agar media, including R2A agar, Luria–Bertani agar (LB; MP Biomedicals), nutrient agar (BD) and tryptic soy agar (BD). Growth of strain BN5^T at different temperatures (5–40 °C at 5 °C intervals) and different pH values (pH 2.0–9.0 at 1.0 pH unit intervals) was tested in R2A broth. Media with pH values below <5.0, 6.0–7.0, and 8.0–9.0 were prepared using sodium citrate, Na_2HPO_4-NaH_2PO_4 and Tris-HCl buffers, respectively, according to the method of Lányí [38], and if necessary, pH values were adjusted after sterilization (at 121 °C for 15 min). NaCl tolerance was determined in R2A broth medium containing different NaCl concentrations (0–5 %, at 1 % intervals), which were manually prepared in the laboratory according to the BD formula. Gram staining was assessed using the Gram stain kit (bioMérieux) according to the manufacturer's instructions. Anaerobic growth was assessed on R2A agar at 25 °C for 3 weeks under anaerobic conditions (with 4–10 % CO_2) using the GasPak Plus system

(BBL). Cell morphology and motility were observed using a transmission electron microscope (JEM-1010; JEOL) and a phase contrast microscope (Zeiss Axio Scope.A1; Carl Zeiss) using cells grown in R2A broth at 25 °C for 2 days, according to previously described methods [39]. Catalase and oxidase activities were assessed as the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide and oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively [40]. The following properties of strain BN5^T and reference strains were investigated in parallel under the same conditions. Hydrolysis of Tween 20, Tween 80, casein, starch, tyrosine and aesculin was assessed on R2A agar according to the methods described by Lányí [38] and Smibert and Krieg [40]. Additional enzymatic activities, biochemical features and oxidation of carbon compounds were tested using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog), respectively, according to the manufacturers' instructions; cells of strain BN5^T and the reference strains resuspended in 0.85 % (w/v) saline were used as inocula for the tests.

Strain BN5^T was capable of degrading all BTEX compounds as well as naphthalene. Strain BN5^T grew well on all the test media, including R2A agar, LB agar, nutrient agar and tryptic soy agar, with optimum growth on R2A agar. Colonies were white to cream coloured, circular and convex, with a diameter of approximately 2–3 mm on R2A agar after 3 days of incubation. Cells of strain BN5^T were Gram-stain-negative, oxidase- and catalase-positive slightly curved rods with a single polar flagellum. The cells were approximately 0.3–0.4 µm wide and 1.1–1.3 µm long (Fig. S2). In a Biolog GN2 MicroPlate, strain BN5^T oxidized sucrose, cellobiose, D-fructose, D-galactose, D-mannose, melibiose, raffinose, trehalose, α-D-glucose, lactose, *N*-acetyl-D-galactosamine, β-methyl-D-glucoside, Tween 80, adonitol, D-arabitol, *m*-inositol, D-mannitol, D-sorbitol, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-glucosaminic acid, α-hydroxy butyric acid, β-hydroxy butyric acid, *p*-hydroxy phenylacetic acid, α-keto butyric acid, α-keto glutaric acid, D,L-lactic acid, malonic acid, propionic acid, quinic acid, sebacic acid, succinic acid, succinamic acid, bromo succinic acid, glucuronamide, D-saccharic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-histidine, hydroxyl-L-proline, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-threonine, D,L-carnitine, γ-amino butyric acid, inosine, uridine, 2-aminoethanol, D,L-α-glycerol phosphate, D-glucuronic acid, *N*-acetyl-D-glucosamine, L-fucose, L-rhamnose, D-gluconic acid, D-glutamic acid, glycyl-L-glutamic acid, L-serine, glycerol, glucose-6-phosphate, D-galacturonic acid, L-arabinose, Tween 40, maltose, methyl pyruvate, mono-methyl-succinate, lactulose, α-keto valeric acid and glucose-1-phosphate, but did not oxidize turanose, 2,3-butanediol, thymidine, itaconic acid, D-psicose, putrescine, *i*-erythritol, phenylethylamine, α-cyclodextrin, dextrin, glycogen,

Table 1. Phenotypic comparisons of strain BN5^T and the type strains of closely related *Paraburkholderia* species

Taxa: 1, strain BN5^T (this study); 2, *P. phytofirmans* KACC 15011^T [13]; 3, *P. dipogonis* LMG 28415^T [14]; 4, *P. insulsa* DSM 28142^T [17]; 5, *P. graminis* DSM 17151^T [12]. All strains are positive for the following characteristics: motility, indole production, catalase, oxidase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-galactopyranosidase activity*, assimilation* of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid, and hydrolysis* of tyrosine, Tween 20 and Tween 80. All strains are negative for the following characteristics: Gram-staining, nitrate reduction*, trypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activity*, and hydrolysis* of casein and starch. +, Positive; –, negative.

Characteristics	1	2	3	4	5
Isolation source	Gasoline-contaminated soil	Onion roots	Root nodules	Arsenic-rich shallow sea	Rhizosphere
Growth at 37 °C	+	–	+	+	+
Glucose fermentation	+	+	+	+	–
Hydrolysis* of:					
Aesculin	+	+	–	+	+
Gelatin	–	–	+	–	–
Enzyme activity (API ZYM)* of:					
Cystine arylamidase, α-chymotrypsin, β-galactosidase and β-glucosidase	–	+	–	–	–
Urease and α-glucosidase	–	–	–	–	+
Lipase (C14)	–	+	–	+	–
Arginine dihydrolase	+	+	+	–	–
Valine arylamidase	+	+	–	+	–
Esterase (C4)	–	+	+	+	+
DNA G+C content (mol%)	62.9†	62.3†	63.2	62.0	62.9†

*These analyses were conducted under the same conditions in this study.

†The DNA G+C contents were calculated based on their genomes in this study.

gentiobiose, xylitol, γ-hydroxy butyric acid, glycyl-L-aspartic acid, L-leucine and urocanic acid (Table S1). Many characteristics of BN5^T, such as the Gram reaction, nitrate reduction, indole production, motility and oxidase and catalase activities, were in good agreement with those of the reference strains of the genus *Paraburkholderia*, whereas other properties, such as growth at 37 °C, hydrolysis of gelatin and aesculin, and many other phenotypic properties differentiated strain BN5^T from other closely related *Paraburkholderia* species (Table 1). The phenotypic characteristics of strain BN5^T are presented in the species description and compared with those of the closely related type strains in Tables 1 and S1.

The isoprenoid quinones of strain BN5^T were analysed with a high-performance liquid chromatography (model LC-20A; Shimadzu) system equipped with a reversed-phase column (250 Í 4.6 mm, Kromasil; Akzo Nobel) and a diode array detector (SPD-M20A; Shimadzu) using methanol–isopropanol (2 : 1, v/v) as an eluent (1 ml min^{-1}), as described by Komagata and Suzuki [41]. To analyse cellular fatty acids, strain BN5^T and the reference strains were cultivated in R2A broth at 25 °C, and then the cells were harvested at the same growth phase (exponential phase, optical density at 600 nm=0.8). The fatty acids were saponified, methylated, extracted and washed using the standard MIDI protocol, and the fatty acid methyl esters were analysed using a gas chromatography system (model 6890; Hewlett Packard) based on the TSBA6 database in the Microbial Identification System (Sherlock version 6.2B) [42]. The polar lipids were analysed by thin-layer chromatography using cells harvested during the exponential growth phase according to a previously described method [43]. The following reagents were used to detect different polar lipids: 10 % ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids) and Dittmer–Lester reagent (for phospholipids). The DNA G+C contents of strain BN5^T and the type strains of *P. phytofirmans* and *P. graminis* were calculated using the EditSeq module of the Lasergene software based on their genome sequences.

Ubiquinone-8 (Q-8) was the predominant respiratory quinone and the major fatty acids (>5 %) of strain BN5^T were $C_{16:0}$ (24.2 %), cyclo-$C_{19:0}\omega8c$ (7.9 %) and summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 7.4 %), which were common to other members of the genus *Paraburkholderia* [13, 14, 17] (Table 2). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified phosphoamino lipid and two unidentified lipids were the major polar lipids, and three unidentified amino lipids and six unidentified lipids were detected as minor polar lipids (Fig. S3). The DNA G+C content of strain BN5^T was 62.93 mol%, which was similar to that of other members of the genus *Paraburkholderia* (Table 1). In conclusion, the phenotypic and chemotaxonomic features of strain BN5^T and the phylogenetic inference support its assignment to a novel species of the genus *Paraburkholderia,* for which the name *Paraburkholderia aromaticivorans* sp. nov. is proposed.

Table 2. Cellular fatty acid composition (%) of strain BN5^T and the type strains of closely related *Paraburkholderia* species

Taxa: 1, strain BN5^T; 2, *P. phytofirmans* KACC 15011^T; 3, *P. dipogonis* LMG 28415^T; 4, *P. insulsa* DSM 28142^T; 5, *P. graminis* DSM 17151^T. All data were from this study. The data are expressed as the percentages of total fatty acids and fatty acids present at less than 1.0 % in all strains are not shown. Major fatty acid components (>5.0 %) are highlighted in bold. –, Not detected; TR, trace amount (<1.0 %).

	1	2	3	4	5
Saturated fatty acid:					
$C_{10:0}$	1.2	TR	TR	1.0	TR
$C_{12:0}$	4.1	**5.0**	3.0	3.4	2.0
$C_{14:0}$	3.2	2.2	3.3	**5.1**	3.7
$C_{16:0}$	**24.2**	**13.1**	**14.8**	**17.8**	**22.4**
$C_{18:0}$	1.4	TR	1.2	1.3	1.7
$C_{19:0}$	3.5	2.6	3.9	2.6	2.8
Branched fatty acid:					
iso-$C_{10:0}$	1.1	TR	TR	TR	TR
iso-$C_{17:0}$	1.8	1.4	1.3	1.4	1.2
anteiso-$C_{15:0}$	1.2	1.3	1.1	1.3	TR
anteiso-$C_{17:0}$	1.1	1.5	1.1	1.4	TR
cyclo-$C_{17:0}$	3.9	3.4	3.6	3.4	**8.4**
Hydroxy fatty acid:					
$C_{16:0}$ 2-OH	1.8	4.7	**6.1**	4.4	3.3
$C_{16:0}$ 3-OH	4.7	**5.8**	**7.6**	**7.1**	**6.3**
$C_{16:1}$ 2-OH	2.2	2.6	2.8	1.7	3.1
$C_{18:1}$ 2-OH	3.2	4.9	3.7	2.8	2.8
iso-$C_{11:0}$ 3-OH	1.9	1.9	1.6	2.1	1.4
Unsaturated fatty acid:					
$C_{12:1}$	2.3	1.3	–	1.4	TR
$C_{20:2}\omega6,8c$	2.5	2.6	2.9	2.7	1.3
iso-$C_{17:1}\omega5c$	1.0	TR	TR	TR	TR
cyclo-$C_{19:0}\omega8c$	**7.9**	**12.2**	**11.8**	**10.3**	**13.1**
Summed feature*:					
1	2.1	1.8	1.6	1.9	1.1
2	4.1	4.7	**5.3**	**7.3**	**5.2**
3	2.0	1.7	1.6	1.3	2.5
5	3.4	2.4	2.9	2.4	2.8
7	1.7	1.6	1.6	1.6	0.9
8	**7.4**	**13.2**	**10.3**	**7.6**	**6.5**

*Summed features represent groups of two or three fatty acids that cannot be separated by gas–liquid chromatography with the MIDI system. Summed feature 1, $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H; summed feature 2, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 5; anteiso-$C_{18:0}$ and/or $C_{18:2}\omega6,9c$; summed feature 7, $C_{19:1}\omega7c$ and/or $C_{19:1}\omega6c$; summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

DESCRIPTION OF *PARABURKHOLDERIA AROMATICIVORANS* SP. NOV.

Paraburkholderia aromaticivorans (a.ro.ma.ti.ci.vo′rans. L. adj. *aromaticus* aromatic, fragrant; L. pres. part. *vorans* devouring; N.L. part. adj. *aromaticivorans* devouring aromatic compounds).

Cells are Gram-stain-negative, facultatively aerobic, catalase- and oxidase-positive, motile, and slightly curved rods with a single polar flagellum. The cells are approximately 0.3–0.4 μm wide and 1.1–1.3 μm long. Colonies on R2A agar are white to cream, circular and convex. Growth occurs at 20–37 °C (optimum, 25–30 °C), pH 3–7 (optimum, pH 5–6) and 0–2 % (w/v) NaCl (optimum, 0 %). Tyrosine, aesculin, Tween 20 and Tween 80 are hydrolysed, but casein, starch and gelatin are not. Does not reduce nitrate. Indole is produced. Glucose is fermented. The cells are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactopyranosidase, arginine dihydrolase and valine arylamidase activities, but negative for trypsin, esterase (C4), lipase (C14), α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, cystine arylamidase, α-chymotrypsin, β-galactosidase, β-glucosidase, urease and α-glucosidase activities. Assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid is positive. Major fatty acids are $C_{16:0}$, cyclo-$C_{19:0}\omega 8c$ and summed feature 8 (comprising $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified phosphoamino lipid and two unidentified lipids are the major polar lipids, and three unidentified amino lipids and six unidentified lipids are the minor polar lipids. Q-8 is the predominant respiratory quinone. The DNA G+C content of the type strain is 62.93 mol %. The type strain is BN5^T (=KACC 19419^T=JCM 32303^T), isolated from gasoline-contaminated soil.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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