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Characterization and Hydrocarbon Degradation Potential of *Variovorax* sp. Strain N23 Isolated from the Antarctic Soil

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Abstract: Increasing pollution has significantly threatened the Antarctic ecosystem. The contamination of hydrocarbons has drawn a considerable amount of attention owing to their toxicity, recalcitrance, and persistence. Considering the Antarctic Treaty, only indigenous species are allowed to bioremediate the contaminated environment. However, the knowledge of the ecological role, physiology, function, and genomics of endemic hydrocarbon consumers is still limited. Here, we investigated the dynamics of phenanthrene-consuming communities derived from the Antarctic soil and found that *Variovorax*, *Rhodocyclaceae*, and *Hydrogenophaga* were differentiated in all the phenanthrene-consuming subcultures. We isolated a pure culture of the key hydrocarbon consumer *Variovorax* sp. strain N23. Moreover, the result of the polyphasic approach suggested that strain N23 represents a novel species of the genus *Variovorax*. In addition, the genomic characteristics of this strain revealed incomplete degradation pathways for diverse hydrocarbons. Overall, this study reveals the relatively weak hydrocarbon-degrading potential of the indigenous bacteria and suggests the need for more careful protection of the Antarctic ecosystem.

Keywords: polycyclic aromatic hydrocarbons; psychrophilic bacteria; genomics; chemotaxis; biodegradation

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1. Introduction

Antarctica is considered the final pristine continent on Earth. However, anthropogenic activities, including scientific research and tourism, have caused increasingly intensive pollution in the Antarctic environment. Petroleum hydrocarbons are ubiquitous pollutants in Antarctica that are derived from accidental oil spills and fuel combustion [1]. Petroleum hydrocarbons mainly consist of n-alkanes, branched alkanes, cycloalkanes, polycyclic aromatic hydrocarbons (PAHs), and other organic substances. Some of these hydrocarbons are of major concern due to their recalcitrance, persistence, and genotoxicity [2]. Previous studies have documented the microbial degradation of hydrocarbons in Antarctic extreme environments [3]. However, knowledge of the ecological role, physiology, function, and genomics of the endemic hydrocarbon consumers is still limited.

The Antarctic Treaty (adopted in 1959 and enforced in 1961) and the Protocol on Environmental Protection to the Antarctic Treaty (adopted in 1991 and enforced in 1998) have placed strict guidelines for the conservation of Antarctic biodiversity and ecosystem, thus prohibiting bioremediation with exotic species. Moreover, Antarctica exhibits unique climatic conditions, including low temperature, high salinity, and strong ultraviolet radiation [4], which are unfavorable to the growth of non-indigenous microbes. By contrast,

native microorganisms adapt well to the Antarctic environment. Therefore, the bioremediation of an oil-contaminated Antarctic environment by indigenous microbes represents a promising approach to mitigate the anthropogenic impact in Antarctica [5,6].

The strains of the genus *Variovorax* are widely distributed in nature, from temperate to Polar regions, and some members are psychrophilic [7,8]. They were isolated from a variety of ecological habitats, including Antarctic lichen, plant rhizosphere, sewage, and contaminated soil with chemicals [9–12]. The genus *Variovorax* is phylogenetically affiliated with the family *Comamonadaceae*, class *Betaproteobacteria* [13]. It exhibits diverse metabolic features. More importantly, it is known for its metabolism of organic pollutants, including PAHs and chlorophenol [7,11]. Recently, Ghimire et al. reported that *Variovorax* sp. PAMC26660, a lichen-associated bacterium isolated from Antarctica, exhibits the capability of aromatic compound decomposition [14]. Therefore, the Antarctic bacteria of genus *Variovorax* are involved in the degradation of aromatic hydrocarbons and represent a promising candidate for application in the bioremediation of the contaminated Antarctic environment. However, the ecological role and hydrocarbon degradation pathways of the Antarctic *Variovorax* strains remain virtually unknown.

In this study, we aimed to (1) investigate the dynamics of phenanthrene-consuming communities enriched from Antarctic soil to determine the key hydrocarbon consumers, (2) isolate, characterize, and classify the pure culture of key hydrocarbon consumers from the phenanthrene-degrading subcultures, and (3) propose putative hydrocarbon degradation pathways in endemic Antarctic bacteria. This study has important implications with respect to the bioremediation of an oil-contaminated Antarctic environment using endemic microbes.

2. Materials and Methods

2.1. Enrichment of Phenanthrene-Degrading Consortia and Isolation of Cultivable Strains

Surface soil (2–3 cm) was collected from the Fildes Peninsula, Antarctica (58°57′51.9″ W, 62°12′59.7″ S). The soil sample was stored in a sterile plastic bag at −20 °C, transported to the lab, and then stored at −80 °C. We enriched the PAH-consuming consortia from the soil using a minimal salt medium [15] supplemented with phenanthrene (0.2 g/L) as the sole source of carbon and energy. Specifically, we dissolved phenanthrene in chloroform, filtered it through a sterile membrane with 0.22 μm pores, and subsequently delivered it to 100 mL of minimal salt medium. The solvent was allowed to evaporate overnight on a shaker before inoculation, as described by Cui et al. [16]. Approximately 5 g of the soil samples were inoculated into the medium. The enrichment of phenanthrene-consuming bacteria was conducted on a rotary shaker (150 rpm) at 25 °C in the dark for approximately 30 days. The treatment without adding soil samples served as the negative control. Next, the enrichment culture (RT0) was transferred to the above-mentioned fresh medium with an inoculum size of 10% (*v*/*v*), repeating this process five consecutive times (RT1–RT5) to produce a robust phenanthrene-degrading consortium. The cultures were prepared in four duplicates for each treatment.

The subculture RT5, after 10^{-4}, 10^{-5}, and 10^{-6} dilutions, was spread onto M8 agar plates [16] and incubated at 25 °C. The colonies with different morphologies were streaked onto fresh M8 plates using sterile toothpicks to obtain pure cultures of cultivable strains.

2.2. DNA Extraction and 16S rRNA Gene Amplicon Sequencing of the Phenanthrene-Degrading Communities

A total of 10 mL of late-exponential phase cultures described in Section 2.1 were centrifuged at 10,000 r/min and 4 °C for 5 min, and microbial DNA was extracted using the E.Z.N.A.® bacterial DNA kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocols. The V3–V4 region of the bacterial 16S rRNA gene was amplified by PCR using universal primers 341F-1/806R-1 (341F 5′-ACTCCTACGGGAGGCAGCAG-3′, 806R 5′-GGACTACHVGGGTWTCTAAT-3′). Thermal cycling consisted of initial denaturation at 94 °C for 3 min, followed by 30 cycles consisting of denaturation at 94 °C for 30 s,

annealing at 56 °C for 45 s, and extension at 72 °C for 30 s, with a final extension of 10 min at 72 °C. Amplicons were purified using the Agencourt AMPure XP magnetic bead and then were added to a tag sequence. Purified amplicons were paired-end sequenced (2 × 250) on an Illumina HiSeq 2500 platform according to standard protocols (BGI Genomics Co., Ltd., Shenzhen, China).

The raw sequence was filtered under specific conditions to obtain high-quality clean reads. The filtered reads merged as tags using FLASH (v1.2.11) [17]. The clean tags were clustered into operational taxonomic units (OTUs) with a 97% similarity cutoff using USEARCH (version 7.0.1090) [18]. Chimeric sequences were removed using UCHIME (version 4.2.40) [19]. Next, the representative OTU sequences were classified using RDP Classifier (version 2.2) [20] against the Greengene database [21], with a confidence threshold of 0.6.

2.3. Biodegradability of Various Hydrocarbons by Strain N23

N-hexadecane (99% purity) was purchased from Kaimike. Cyclohexane (99.5% purity) and methylcyclohexane (99% purity) were purchased from Aladdin (Shanghai, China). Dodecylcyclohexane (98% purity) was purchased from Tokyo Chemical Industry (Nihonbashi-honcho, Chuo-ku, Tokyo). Naphthalene (95% purity) was purchased from Rhawn (Shanghai, China). Phenanthrene (97% purity) was purchased from Macklin (Shanghai, China). Salicylic acid (≥98% purity) was purchased from Solarbio (Beijing, China).

To determine the hydrocarbon utilization range of strain N23, an incubation experiment was conducted in 100 mL of the ONR7a medium supplemented with each of the above-mentioned hydrocarbons (each at 0.1 g/L) as the sole source of carbon and energy in duplicate. The late-exponential phase culture of strain N23 was inoculated into the medium with an inoculum size of 1% (*v*/*v*). The cultures were then incubated in the dark at 20 °C on a rotary shaker (150 rpm) for 14 days. The control treatment without any carbon source was conducted in parallel. We measured the optical density (OD_{600}) of the cultures to determine the bacterial growth and hydrocarbon degradation.

2.4. Whole Genome Sequencing and Analysis of Strain N23

The genomic DNA of strain N23 was extracted using the standard method [22]. The quantity of extracted DNA was detected using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA). The completeness of DNA was examined using 1% (*w*/*v*) agarose gel electrophoresis. The genomic DNA was sequenced using whole-genome shotgun technology via the Illumina NovaSeq platform at Personal Biotechnology Co., Ltd. (Shanghai, China). The library was prepared using the TruSeq™ DNA sample prep kit (Illumina, San Diego, CA, USA). Total raw reads were generated and subjected to quality control by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/, accessed on 10 February 2020). Next, the obtained high-quality reads were assembled using A5-MiSeq (https://arxiv.org/abs/1401.5130, accessed on 10 February 2020) [23] and SPAdes (http://cab.spbu.ru/files/release3.12.0/manual.html, accessed on 10 February 2020) [24]. The genome sequence of strain N23 was deposited at GenBank under accession number JAOOPY000000000. Three types of functional elements were analyzed, including protein-coding sequences (CDSs), non-coding RNA (ncRNA), and clustered regularly interspaced short palindromic repeats (CRISPRs). The CDSs were predicted using GeneMarkS (http://topaz.gatech.edu/GeneMark/, accessed on 20 March 2020) [25]. The tRNA and rRNA were calculated using tRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/, accessed on 20 March 2020) [26] and Barrnap (http://www.vicbioinformatics.com/software.barrnap.shtml, accessed on 20 March 2020), respectively. CRISPR finder (http://crispr.i2bc.paris-saclay.fr/Server/, accessed on 20 March 2020) [27] was used to predict the CRISPRs. All genes of strain N23 were annotated using blasting against multiple databases, including NCBI NR, COG, KEGG, Swiss-Prot, and GO.

An online digital DNA–DNA hybridization (dDDH; https://ggdc.dsmz.de, accessed on 15 July 2022) tool based on a genome-to-genome sequence comparison was used for

microbial species delineation, thus reliably mimicking conventional DDH. Specifically, the genome–genome distance calculator (GGDC) compared the genome of strain N23 with those of its closest related type strains, calculating an inter-genomic distance using three distinct formulas. These distances were transformed to values analogous to DDH using a generalized linear model (GLM) inferred from an empirical reference dataset comprising real DDH values and genome sequences [28]. Additionally, to further elucidate if strain N23 represents a new species of the *Variovorax* genus, the average nucleotide identity (ANI) value was calculated using the EzBiocloud ANI calculator (https://www.ezbiocloud.net/tools/ani, accessed on 15 July 2022), which adopts the OrthoANIu algorithm to classify and identify bacteria [29].

2.5. Phylogenetic Analysis of Strain N23

The 16S rRNA gene nucleotide sequence of strain N23 was aligned with the published sequences in GenBank using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 1 May 2022). Then, phylogenetic analysis of strain N23 and its closely related type strains was conducted based on the multiple sequence alignment of the data using MEGA software (version 7). The evolutionary distances were computed using the Tamura-Nei method [30], reported as the number of base substitutions per site. Evolutionary clustering was inferred using the neighbor-joining algorithm [31] and determined using bootstrap values based on 1,000 replicates. The 16S rRNA gene of this strain is available under GenBank database accession number OP503480.

To verify the taxonomic position of strain N23, a genome-based phylogenomic tree was inferred using GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database [32,33]. We conducted multiple sequence alignment and concatenation analyses to describe the phylogeny of strain N23 based on a total of 120 ubiquitous single-copy inherited proteins extracted from strain N23 and other previously described species of the genus *Variovorax*.

2.6. Statistical Analysis and Data Visualization

Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance was performed to visualize the divergence between the enrichment culture and subcultures. An analysis of similarity (ANOSIM) was performed to evaluate whether there were significant differences among the above groups. Generally, a P value = 0.05 was regarded as statistically significant. The above analyses were performed using the Biozeron Cloud Platform (http://www.cloud.biomicroclass.com/CloudPlatform, accessed on 9 October 2022). In addition, a bubble image of indicator species was visualized in R with an indicspecies package. The pathways of chemotaxis and motility were adopted from the KEGG database. GraphPad Prism (version 9) was used for processing and visualization with DDH and ANI data.

3. Results and Discussion

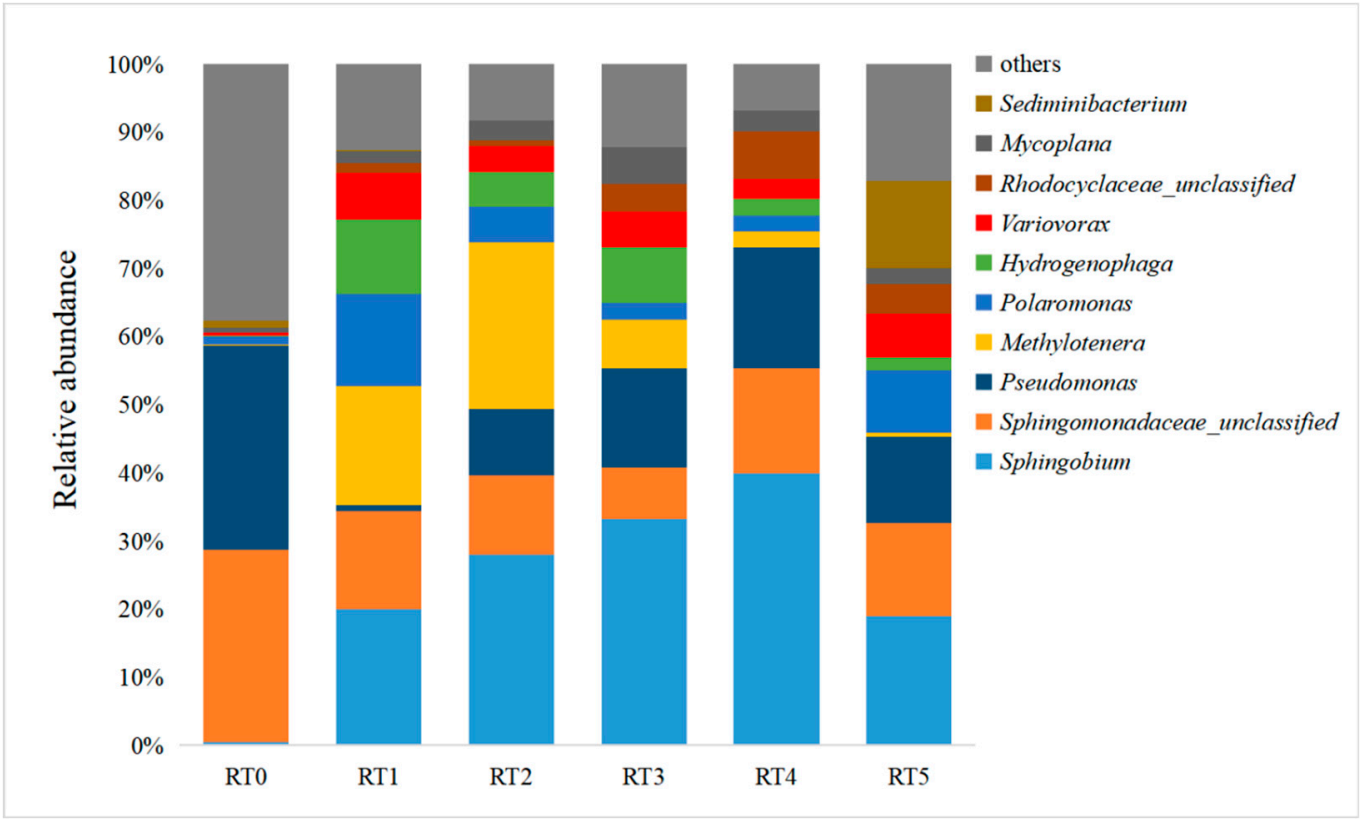
3.1. The Dynamics of Phenanthrene-Degrading Communities Derived from Antarctic Soil

NMDS analysis was performed to visualize the divergence of microbial communities between enrichment culture and subcultures (Figure S1). Remarkably, the enrichment culture (RT0) formed a separate cluster on the plot, while all the subcultures (RT1–RT5) clustered with each other. Moreover, the result of ANOSIM further demonstrated that the enrichment culture distinguished itself from all the subcultures ($P < 0.05$), while the discrepancy between pairwise subcultures was not significant ($P > 0.05$) (Table S1).

We observed a noticeable shift in bacterial species composition from the enrichment culture to the subcultures (Figure 1a). More specifically, *Pseudomonas* and *Sphingomonadaceae_unclassified* were dominant in phenanthrene-consuming enrichment culture (from higher to lower relative abundance, 30.0–28.3%). By contrast, the dominant species in the subcultures exhibited a different profile to that of the enrichment culture. *Sphingobium*, *Sphingomonadaceae_unclassified*, *Pseudomonas*, *Methylotenera*, *Polaromonas*, *Hydrogenophaga*,

and *Variovorax* were dominant in the phenanthrene-consuming subcultures (from higher to lower relative abundance, 28.1–5.0%).

Consecutive incubation with phenanthrene as the sole carbon source eliminated those non-PAH consumers while enriching those PAH consumers. *Variovorax*, *Rhodocyclaceae*, and *Hydrogenophaga* were differentiated in all the phenanthrene-consuming subcultures (Figure 1b), suggesting their pivotal role in PAH decomposition. We successfully isolated strain N23 from the subcultures. Preliminary phylogenetic analysis showed that it belonged to the genus *Variovorax*, which is one of the dominant species in the Antarctic phenanthrene-consuming community (Figure 1a).



(**a**)

Figure 1. *Cont.*

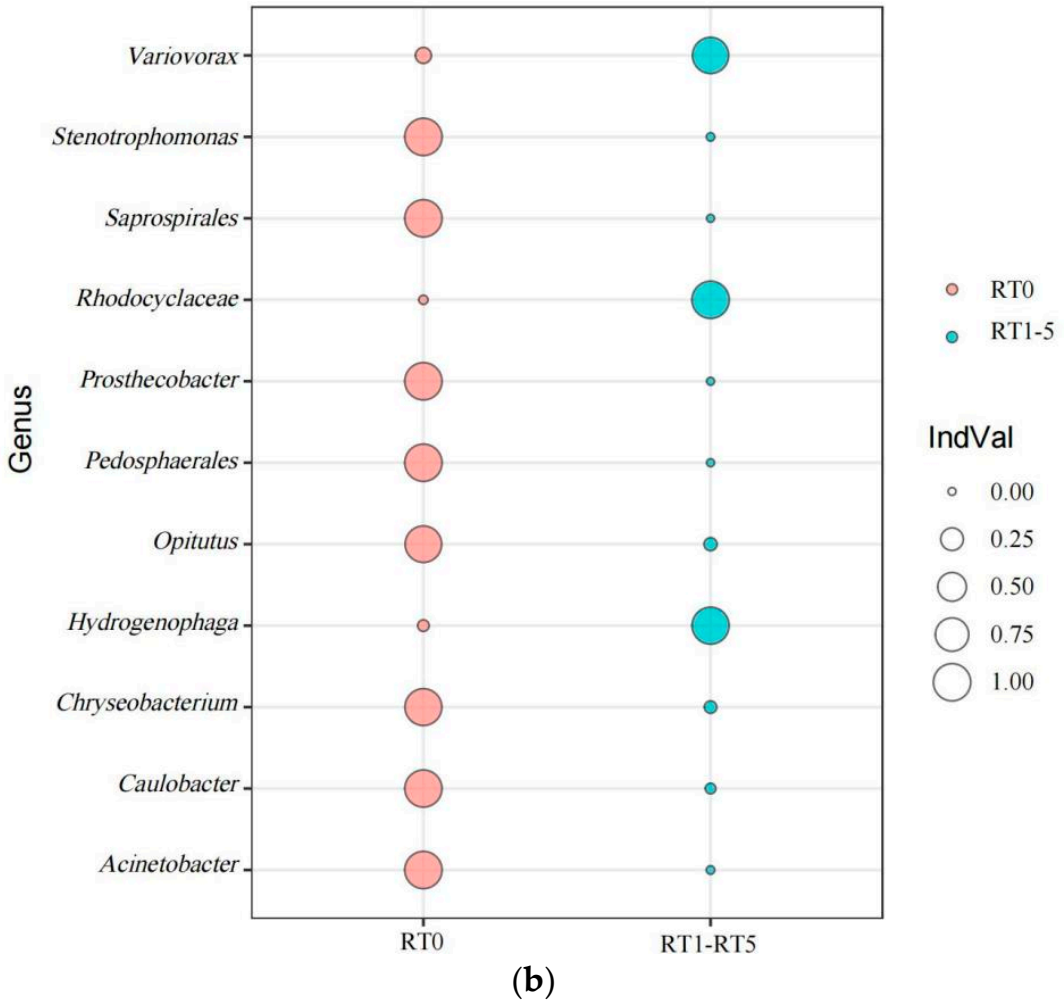


Figure 1. Key players involved in phenanthrene degradation. RT0, phenanthrene-consuming enrichment culture. RT1–RT5, phenanthrene-consuming subcultures. (**a**) Dynamics of phenanthrene-consuming bacterial communities derived from Antarctic soil. The relative abundance of the top 10 most abundant genera is presented. (**b**), Differentiation of key taxa enriched from phenanthrene-consuming subcultures derived from Antarctic soil. Differentiated genera of the phenanthrene-spiked communities by cross-validation analysis. The Y-axis denotes the differentiated taxa of the phenanthrene-spiked communities. The X-axis denotes different treatments. The circle size denotes the indicator value of the corresponding microbial taxa.

3.2. Phylogeny of Strain N23 and Proposal of a Novel Species

Based on the 16S rRNA gene sequence analysis, strain N23 exhibited the highest similarity (99.12%) to the type of strain *Variovorax boronicumulans* NBRC 103145^{T} [11]. Moreover, a phylogenetic tree based on the multiple sequence alignment of the 16S rRNA gene sequences also revealed that strain N23 belongs to the genus *Variovorax* and formed a monophyletic clade with the closet type strain *V. boronicumulans* NBRC 103145^{T} (Figure 2a). Similarly, strain N23 formed an independent branch distinct from other reference species of the genus *Variovorax* in the phylogenomic tree (Figure 2b).

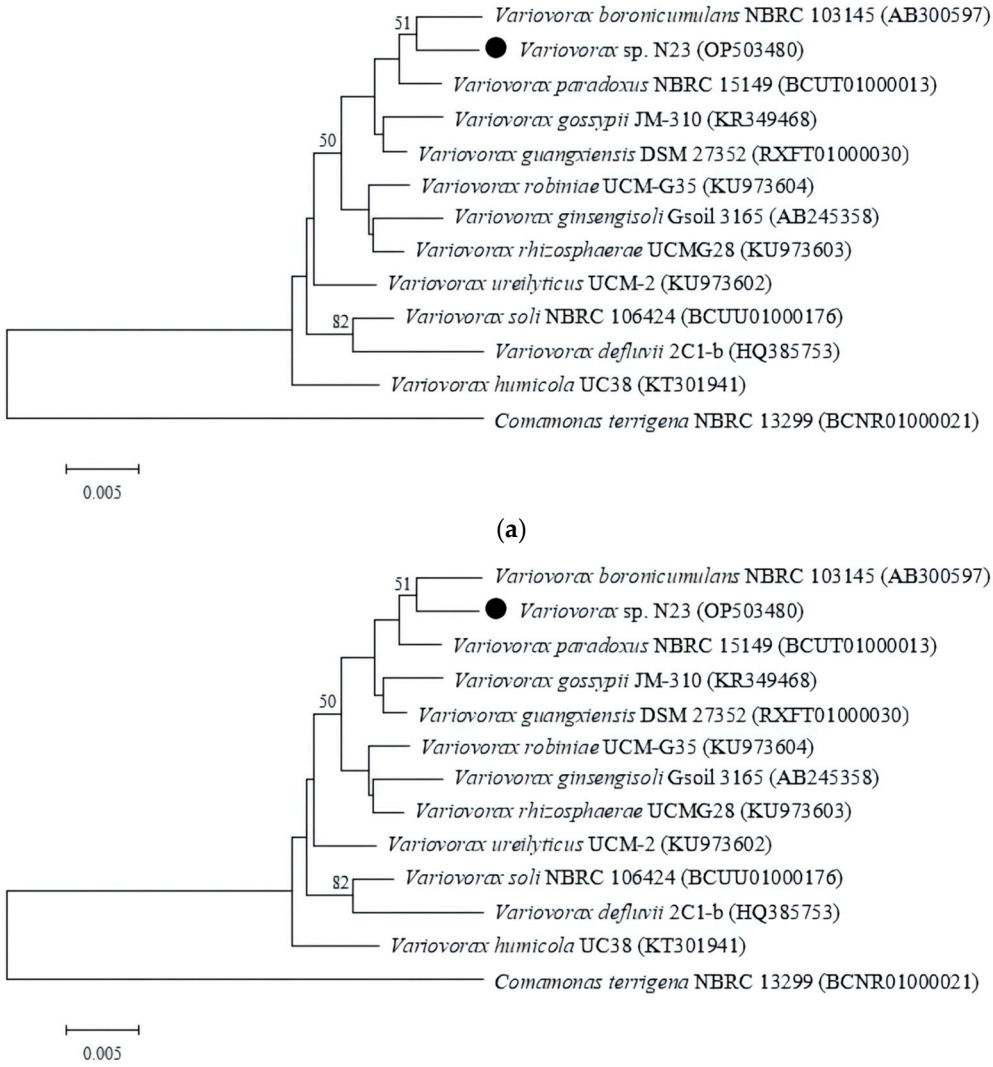


Figure 2. Phylogenetic relationship of strain N23 with its closely related strains. (**a**) A neighbor-joining tree illustrating the phylogenetic relationship of strain N23 and its closely related type strains of genus *Variovorax* constructed based on the 16S rRNA gene sequences. The numbers at branch points indicate bootstrap percentages based on 1000 replicates. Only values > 50% are shown. The genus *Comamonas*, affiliated with the same family *Comamonadaceae*, was selected as the outgroup. (**b**) The phylogenomic tree of strain N23 and its reference species of genus *Variovorax*.

For species delineation, the accepted boundaries of DDH and ANI are 70% and 95–96%, respectively [34,35]. According to the dDDH result calculated using the three formulae, strain N23 exhibited a low similarity (24.4–65.3%) compared to its closely related type species of genus *Variovorax* with available genomes (Figure 3). Moreover, the calculated ANI values (80.3–94.7%) between strain N23 and its closely related type strains are below the cutoff values for the discrimination of a novel species. In brief, we suggested that strain N23 probably represents a novel species of the genus *Variovorax*. However, detailed analyses are needed to justify our inference.

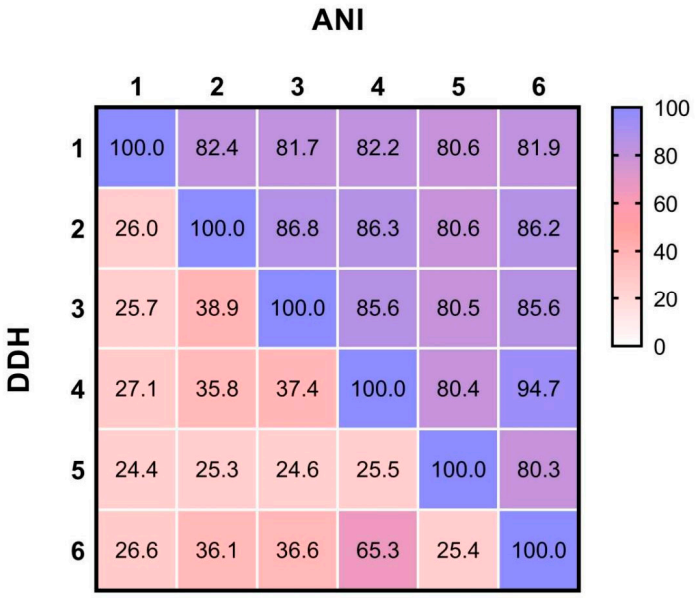


Figure 3. Heatmap of DDH and ANI percentages between strain N23 and its closely related type strains of genus *Variovorax*. Strain 1, *Variovorax* sp. strain N23; 2, *V. boronicumulans* NBRC 103145^{T}; 3, *V. guangxiensis* DSM 27352^{T}; 4, *V. paradoxus* NBRC 15149^{T}; 5, *V. soli* NBRC 106424^{T}; 6, *V. beijingensis* 502^{T}.

3.3. Hydrocarbon Utilization Profile of Strain N23

Strain N23 was a dominant species in phenanthrene-degrading subcultures derived from the Antarctic soil (Figure 1). However, it failed to grow on any of the abovementioned hydrocarbons as a sole source of carbon and energy, as described in Section 2.3. We speculated that strain N23 probably co-metabolizes PAHs with other microbes in the bacterial communities. Therefore, strain N23 exhibited no degradation of hydrocarbons, especially when it was in pure culture. Vasileva et al. also found that some dominant species of pure culture isolated from oil-contaminated Antarctic soils failed to grow on hydrocarbon as a sole source of carbon and energy [36]. By contrast, the hydrocarbon-consuming bacterial consortium usually demonstrates advantages over hydrocarbon consumers in a pure culture, which might be ascribed to the biochemical synergistic effect between the strains of the bacterial consortium. For instance, we found the defined consortium of *Cycloclasticus* sp. PY97M and *Marinobacter* sp. D15-8W exhibited higher PAH degradation rates than the pure culture of PAH-consuming *Cycloclasticus* strains [16]. Therefore, harnessing bacterial consortium represents a more promising strategy for cleaning up the hydrocarbon contamination in Antarctica.

3.4. General Genomic Characteristics of Variovorax sp. Strain N23

The library generated 9,830,392 high-quality reads, accounting for 99.02% of the total reads. Strain N23 has a genome size of 5.29 Mb with a G + C content of 67.89% (Table 1, Figure S2). We assembled 47 contigs and 45 scaffolds, respectively. The length of the largest scaffold was 587,210 bp. The genome of strain N23 was annotated for 5010 CDSs and 2 CRISPR elements. Moreover, 6 rRNA genes (two 5S rRNA genes, two 16S rRNA genes, and two 23S rRNA genes) and 48 tRNA genes were predicted in the genome of strain N23.

Table 1. Genomic features of *Variovorax* sp. strain N23.

Genomic Features	Values
Genome size (bp)	5,292,575
G + C content (%)	67.89
Number of contigs	47
Number of scaffolds	45
Scaffold N50 (bp)	319,074
Scaffold N50 number	7
Max sequence length (bp)	587,210
Min sequence length (bp)	1077
Coding sequences	5010
CDS/total genome (%)	90.25
CRISPR elements	2
Non-coding RNA (ncRNA)	73
ncRNA/total genome (%)	0.24
rRNA	6 (5S, 16S, 23S)
tRNA	48

In total, 4484 (89.50%), 2337 (46.65%), 4757 (94.95%), 3878 (77.41%), and 3665 (73.15%) genes were annotated by COG, KEGG, NR, GO, and Swiss-Prot, respectively. For instance, the distribution of functional genes was evaluated using the COG database (Figure S3). The predominant classes of COGs had an unknown function (22.97%), followed by amino acid transport and metabolism (8.78%), inorganic ion transport and metabolism (7.01%), transcription (6.79%), and energy production and conversion (5.95%) when normalized to the total number of entries.

The metabolism pathways were also predicted based on KEGG analysis. The complete pathways for glycolysis/gluconeogenesis, as well as the citrate cycle and pentose phosphate pathway, were identified in strain N23. Moreover, a series of biosynthesis pathways for amino acids, fatty acids, and vitamins were almost complete. However, this strain harbors incomplete degradation pathways for diverse hydrocarbons, including ethylbenzene, toluene, naphthalene, and other PAHs, implying its relatively weak activity to consume these compounds.

3.5. Ecological Adaptability of Variovorax sp. Strain N23 to the Antarctic Environment

Strain N23 harbors abundant chemotaxis and flagella-assembled protein-encoding genes (17 genes and 35 genes annotated by KEGG, respectively). Chemotaxis and motility are considered bioavailability-promoting features for hydrocarbonoclastic bacteria [37]. In strain N23, methyltransferase CheR and methylesterase, CheB catalyzes the methylation and demethylation of the methyl-accepting chemotaxis proteins (MCPs) and eventually affects the flagellar rotation (Figure 4) [38]. A two-component system is the core of the chemotaxis regulatory protein family; the system consists of a histidine kinase CheA as the sensor and a response regulator CheY [39]. Additionally, the chemotaxis protein CheW connects MCPs to CheA and is essential for the chemotactic signal transduction process in numerous bacteria [40]. When the sensor CheA is activated by the autophosphorylation of its histidine residue, the response regulator CheY is phosphorylated when switching on the rotor of the flagella motor [41]. The chemotaxis and motility processes enable the strain to forage for carbon sources (e.g., hydrocarbons) and move in the high fluid viscosity in Antarctica's low-temperature environment. In addition, the strain also harbors gene clusters that encode the biosynthesis of lipopolysaccharides and unsaturated fatty acids, thus protecting the bacterial cells from the low temperature and enhancing the cytomembrane fluidity [42]. In summary, the abovementioned features enable strain N23 to adapt to the extreme environment in Antarctica and gain an ecological niche advantage over its counterparts.

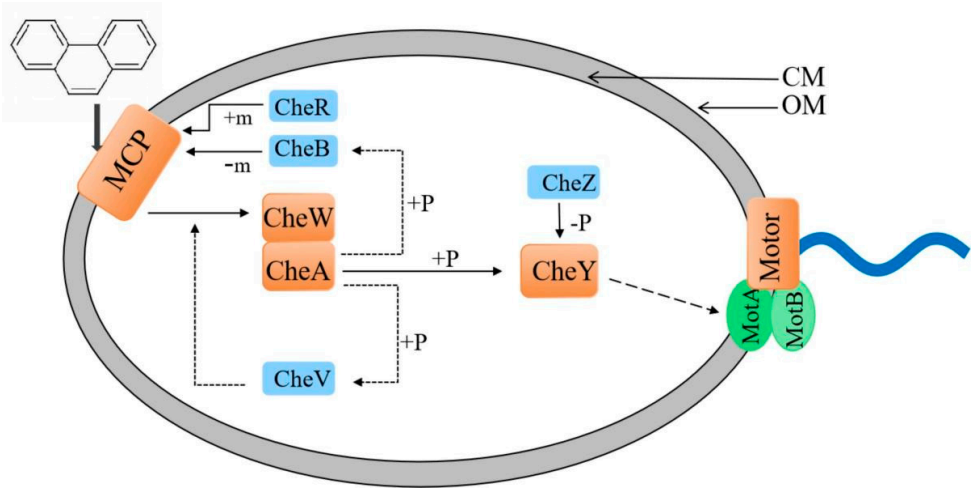


Figure 4. Chemotaxis and motility of *Variovorax* sp. strain N23. The chemotaxis and motility genes are predicted by the KEGG database. +m—methylation; −m—demethylation; +P—phosphorylation; −P—dephosphorylation; OM—outer membrane; CM—cytoplasm (inner membrane). The solid and long dotted arrows represent one-step and multistep reactions, respectively. The short, dotted arrows represent feedback regulation.

3.6. Identified Genes Encoding Hydrocarbon Metabolism

A series of genes related to the metabolism of PAHs and aromatic compounds were identified in the genome of strain N23. Specifically, four genes of naphthalene 1,2-dioxygenase components were annotated in the genome by both blastNR and Swiss-Prot. Naphthalene dioxygenase (NDO) is a member of the bacterial aromatic-ring-hydroxylating dioxygenase (ARHD) family and catalyzes the initial step of PAH degradation by adding two molecular oxygens into aromatic rings [43]. It comprises multicomponent enzymes, including a terminal oxygenase, a ferredoxin, and a flavoprotein [44].

Salicylate hydroxylase-encoding genes are also found in the genome of strain N23 by KEGG annotation. Salicylate is a key intermediate substance in the degradation of PAHs, including naphthalene and phenanthrene [45]. Salicylate was catalyzed to catechol by salicylate hydroxylase, which entered into the downstream PAH degradation pathway. In addition, both genes encoded phthalate 4,5-dioxygenase, and protocatechuate 3,4-dioxygenase was annotated in the genome, indicating the presence of two different downstream degradation pathways through salicylate and phthalate, respectively [45]. However, all the above-mentioned pathways were incomplete, indicating that the strain N23 might co-metabolize with the PAHs of other microbes.

Previous studies revealed that chemotaxis plays a pivotal role in sensing alkanes [40]. The MCP system is indispensable for chemotaxis by hydrocarbonoclastic bacteria. As mentioned previously, MCP-encoding genes were abundant in strain N23, implying the presence of metabolic genes for alkanes. Using KEGG annotation, we identified *ladA*, which encodes a long-chain alkane monooxygenase [46]. We also annotated cytochrome P450: a monooxygenase that catalyzes alkane degradation [47]. It exhibited the highest similarity to its counterparts in *Acidovorax* sp. JS42 (93.3% AA similarity) and *Rhodococcus erythropolis* (55.8% AA similarity) by blastNR and the Swiss-Prot database, respectively. Moreover, we annotated the NAD(P)-dependent alcohol dehydrogenase and aldehyde dehydrogenase encoding genes involved in alkane degradation, which dehydrogenates the primary alcohol to the corresponding aldehyde and oxidizes aldehyde to the fatty acid. Finally, a putative complete pathway for the beta-oxidation of the fatty acids was identified, indicating that the strain N23 might be a versatile hydrocarbon consumer.

4. Conclusions

Indigenous hydrocarbon degraders are significant for the bioremediation of oil-polluted Antarctic environments. We found that strains of the genus *Variovorax* were dominant and differentiated in the phenanthrene-consuming communities. However, the pure culture of the *Variovorax* sp. strain N23 exhibited no catabolic activity toward various hydrocarbons, which might be ascribed to its incomplete degradation pathways for diverse hydrocarbons in the genome. In conclusion, this study sheds light on the ecological role and function of endemic hydrocarbon consumers and suggests the additional careful protection of the unique Antarctic environment from hydrocarbon contamination.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres14010009/s1, Figure S1: The dissimilarity of phenanthrene-consuming bacterial community structures by NMDS analysis; Figure S2: Circular view of the genome of *Variovorax* sp. strain N23 visualized using CGViewer; Figure S3: COGs present in *Variovorax* sp. strain N23; Table S1: Analysis of similarities (ANOSIM) between bacterial community structures from different treatments.

Author Contributions: Conceptualization, J.L.; methodology, J.L. and T.H.; visualization, J.L. and X.L.; formal analysis, J.L., T.H., Y.L., X.L. and K.F.; data curation, J.L.; writing, original draft preparation, J.L.; writing, review and editing, Z.C.; supervision, Z.C. and L.Z.; project administration, Z.C.; and funding acquisition, Z.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data generated during the study is contained within the article.

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Conflicts of Interest: The authors declare no conflict of interest.

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