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Molecular identification of Antarctic canola oil-degrading bacteria

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Abstract: The detrimental impacts of human activity in Antarctica can pose significant threats to the continent's ecosystems and diversity. Various pollutants have been detected in Antarctica, including hydrocarbon compounds derived from oils. The widespread use of canola oil in catering at Antarctic research stations raises the possibility of its release into the environment, either through accidental spillage or via waste cooking oil present in grey water. To help address this issue, a bacterial consortium (reference BS14) obtained from the natural Antarctic environment was isolated and confirmed to be capable of breaking down canola oil. The identity of members of the consortium was investigated using metagenomic analysis, with lipase-producing bacteria further examined using Sanger sequencing. The consortium in media not exposed to canola oil consisted primarily of Proteobacteria and Firmicutes in almost equal percentages. After being exposed to either fresh or waste canola oil, high proportions of representatives of Pseudomonadaceae and Carnobacteriaceae were present. Amongst the bacterial taxa identified in the metagenomic analysis, representatives of the genera *Pseudomonas* and *Carnobacterium* were confirmed to be responsible for biodegrading waste canola oil and pure canola oil. This study offers novel insights into the potential of bacterial consortia for canola oil bioremediation in Antarctica.

Keywords: Antarctic, O'Higgins Station, biodegradation, Sanger sequencing, lipase-producing bacteria.

Introduction

Until the last two centuries, Antarctica was not subjected to anthropogenic activity and human settlement due to its remote location and lack of accessible resources. Human exploration began in the 19th century and, in recent decades, the continent has become a hub for scientific research. Since the latter part of the 20th century, increasing numbers of tourists have been attracted to the unique wildlife and landscapes of Antarctica, leading to a potential rise in adverse effects on the local environment (Tin *et al.* 2009; IAATO 2021).

The Antarctic environment faces contamination from three main sources: global pollution, local waste and fuel issues, and human activities related to research and tourism (Szopinska *et al.* 2017). Among these, chemical contaminants, sewage, grey water disposal and oils, including gasoline, fuel, biological fuel and lubricants, are major contributors to pollution in Antarctica. Oil spills, which occur

frequently in various parts of the continent, have the potential to cause significant harm to its fragile ecosystems (Bharti *et al.* 2016).

Despite considerable attention in the Antarctic Treaty System towards waste management, including wastewater treatment, waste management planning and effective waste practices, instances of accidental release, as well as deliberate waste dumping, have been reported (COMNAP 2006). Such incidents highlight the ongoing challenges posed by both land-based and ship-generated waste. Anthropogenic activities have been identified as a key factor leading to oil-related hydrocarbon spills, constituting long-term threats to ecosystems and their component biodiversity (Koshlaf and Ball 2017; Miri *et al.* 2018).

Canola oil is a widely used vegetable oil owing to its fatty acid composition and affordability (Parcell *et al.* 2018). Its physical properties are maintained at low temperatures, with a freezing point of −10°C a property attributed to its high unsaturated fatty acid content. When heated, vegetable oils, particularly those rich in unsaturated fatty acids, can undergo chemical changes, leading to oxidative stability issues and potentially harmful compound formation (Kumar *et al.* 2012a, 2012b). Consumption of deep-fried foods, containing oxidized compounds such as acrylamide, has raised health concerns (Ananey-Obiri *et al.* 2018).

Oil pollution events can have detrimental effects on biota, including oiling of animals, oxygen depletion and mortality of sessile organisms (Li *et al.* 2015; Puasa *et al.* 2021). Oil persistence in cold environments, where natural remediation processes are slowed, is a key issue. Impacts of oil pollution include acute toxicity, mechanical harm and long-term persistence, affecting a wide range of marine and terrestrial organisms (EUROWA 2019; NOAA 2019).

Environmental remediation involves removing pollutants sourced from human activities, often relying on microorganisms for bioremediation (Zamree *et al.* 2023). Microbes can break down contaminants into harmless compounds, with lipases being critical enzymes for breaking down longer fatty-acid chains present in oils such as canola oil (Park and Park 2022). Antarctic representatives of bacterial genera, including *Rhodococcus*, *Pseudomonas* and *Arthrobacter*, have demonstrated potential for oil degradation (Sampaio *et al.* 2017; Lee *et al.* 2018; Roslee *et al.* 2020; van Dorst *et al.* 2021).

Metagenomics provides an approach for examining the diversity, ecology and functions of microorganisms in their natural environments, and has transformed microbiology by allowing exploration of difficult-to-culture microorganisms, also facilitating bioremediation efforts (Bashir *et al.* 2014; Junemann *et al.* 2017; Amrane and Lagier 2018). It offers insights into microbial processes and can guide the design of microbial communities for effective degradation and the construction of databases documenting the specialized abilities of microbial strains (Nazir 2016; Malla *et al.* 2018). In this context, the present study aims to examine the bacterial consortium involved in the degradation of canola cooking oil by (*i*) employing metagenomic analysis to assess the overall microbial diversity, and (*ii*) isolating culturable bacterial strains responsible for degradation, which were subsequently identified through Sanger sequencing.

Materials and Methods

An unknown and diverse Antarctic bacterial consortium, coded BS14, was originally isolated from soil near the Chilean General Bernardo O'Higgins Riquelme research station, located on the northwestern Antarctic Peninsula (Zahri *et al.* 2021). Approximately 15 g of soil, collected from a depth of 2 to 5 cm, was placed in a sterile tube. The sample was kept at −20°C for up to two months, including during transportation to Malaysia. Waste canola oil (WCO) and pure (fresh) the canola oil (PCO) (produced by Belmont, Chile) were obtained from the research station kitchen. WCO refers to the used and excess oil generated after each cooking process at the station. Both WCO and PCO are stored at room temperature at the facility. No specific data or records are available regarding the frequency of WCO use or its exact applications in cooking. However, various methods of food preparation, including frying, grilling and boiling are routinely used.

Preparation of growth medium for bacterial consortium

Detailed methods for bacterial enrichment and growth medium preparation can be found in a work by Zahri *et al.* (2021). Biodegradation of WCO and PCO by consortium BS14 has previously been selected due to its highest biodegradation capabilities and has been optimised using response surface methodology (RSM).

The RSM optimised medium consisted (per L) of 8.34 g K_2HPO_4, 2.61 g KH_2PO_4, 0.13% w/v NaCl, 1 g $(NH_4)_2SO_4$, 1.13 g yeast extract and 1% v/v WCO with the pH adjusted to 7.13 using HCl. The medium for PCO consisted of (per L) 9.52 g K_2HPO_4, 2 g KH_2PO_4, 0.75 g $(NH_4)_2SO_4$, 1 g yeast extract and 1% PCO, with the pH adjusted to 7.25 using HCl. A 1 ml aliquot of culture was inoculated into 50 mL of both media and incubation took place on an orbital shaker (150 rpm) at 10°C. The bacterial consortium was standardised at an optical density (OD 600 nm) of 1.00 ± 0.1.

Identification of Antarctic bacterial consortium (BS14) through metagenomic analysis

Samples were identified through metagenomic analysis, including the bacterial consortium from the original culture (OC), as well as those treated with waste canola oil (WT) or pure canola oil (PT), where the "T" in WT and PT indicated "treated". The OC refers to the enriched bacterial community obtained from Antarctic soil, which was previously shown to have oil-degrading potential. Waste canola oil (WCO) is used cooking oil, while pure canola oil (PCO) is fresh, unused oil.

DNA extraction for full-length bacterial 16S rRNA

Total genomic DNA was extracted from each sample (OC, WT and PT) using the Macherey-Nagel microbial Nucleospin® DNA Extraction Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. DNA from the WT and PT was extracted from the RSM optimised medium on the third day of the degradation process to ensure that all bacteria present at the start of primary-stage biodegradation were identified. DNA from the OC was extracted from nutrient broth (NB) cultures after 2 days of incubation on an orbital shaker at 10°C and 150 rpm. Ten milliliters of bacterial culture was centrifuged at 4 436 × *g* (RCF or Relative Centrifugal Force) for 10 min. Then, 20 mg of the resultant pellet was resuspended in 100 µL of elution buffer (EB) for the extraction process.

The extracted genomic DNA samples were diluted to 100 ng µL^{-1} and mixed with 1 µL of loading dye. All samples were run on 1% (w/v) agarose gel in 1X Tris-acetate-EDTA (TAE) buffer at 80 V for 1 h. The gel was then visualised using a UV transilluminator (BTLab System, USA). The genomic DNA was analysed using the VC 1-kb Ex DNA ladder (Vivantis, USA) as size standard. DNA samples were stored at −20°C until further analysis (Pootakham *et al.* 2017).

16S rRNA analysis of bacterial consortium

The full-length 16S rRNA gene was amplified using the polymerase chain reaction (PCR) with specific containing barcodes and 5' phosphate modifications to facilitate single-molecule real-time (SMRT) sequencing, as described by Pootakham *et al.* (2017). The primers used were 27F (/5Phos/AGAGTTTGATCCTGGCTCAG) and 1492R (/5Phos/GNTACCTTGTTACGACTT), each incorporating a 5' buffer sequence (GCATC) to improve sequencing efficiency. The PCR master mix was prepared by combining 1.5 µL of PCR-grade water and 12.5 µL of 2X KAPAHiFi™ Hotstart ReadyMix (Treecode, Singapore). Subsequently, the master mix was mixed with 5.4 µL of the barcoded forward primer (2.5 µM) and 3 µL of the barcoded reverse primer (2.5 µM). To this, 5 µL of diluted gDNA sample (input gDNA 25–100 pg) was added, and the mixture was gently mixed and centrifuged at 4°C to ensure the reaction mixture settled at the bottom of the tube.

The PCR program consisted of an initial denaturing step at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 s and extension at 72°C for 1 min. The quality of the PCR products was evaluated using 2% (w/v) agarose gel electrophoresis and an Agilent Bioanalyzer to confirm the correct size and concentration of the amplicons.

Purification, library construction and sequencing of DNA samples

PCR products were purified using the Qiagen gel extraction kit (Qiagen, Gemany). A DNA-binding enzyme was used to ligate sequencing linkers to the amplified DNA fragments, enabling the construction of a SMRTbell™ library. The resulting fragments were further purified using AMPure® PB magnetic beads. Prior to use, the beads were brought to room temperature and mixed thoroughly to ensure a homogeneous suspension. Beads were added to the sample tube, followed by elution buffer to bring the volume to 100 µL. Tubes were centrifuged briefly and then placed on an end-over-end rotator for 10 min to bind the DNA to the beads. After centrifugation, cleared supernatant was pipetted and transferred. Beads were washed with ethanol, dried and eluted with EB. DNA concentration was measured using Qubit fluorometer (Thermo Fisher, USA).

SMRTbell™ library construction consisted of three main steps: DNA damage repair, end-repair, and adapter ligation. The PCR product was first diluted to 10.6 ng µL^{-1} and incubated with a prep buffer, NAD and DNA damage repair mix v2 (used for DNA ligation process). End-repair followed, with incubation at 20°C for 30 min and 65°C for another 30 min. Adapter ligation was then carried out at 20°C for 60 min. The resulting SMRTbell™ template underwent two rounds of purification using AMPure® PB beads. The final library was quantified using a Qubit fluorometer, and insert size was determined using the Agilent 2100 Bioanalyzer. DNA quality was also checked via 2% (w/v) agarose gel electrophoresis before sequencing on the PacBio platform.

To create a Single-Molecule-Real-Time Bell (SMRT bell™) library, the process involves generating appropriately sized double-stranded DNA fragments. This can be accomplished through random DNA shearing or targeted amplification of specific regions. The SMRTbell™ library is assembled by attaching universal hairpin adapters to these fragments. Any hairpin dimers formed during the attachment process are removed using size-selective magnetic bead purification, aided by PacBio's MagBead kit. The final step includes using exonucleases to eliminate unsuccessful ligation products. Following exonuclease treatment and AMPure® PB purification, the sequencing primer is introduced to the SMRTbell™ templates, facilitating sequence binding.

The resulting SMRTbell™ library was then sequenced on the PacBio Sequel® II System, considering its high throughput, cost-effectiveness, high fidelity (> 99% accuracy) and long-read capabilities (up to 500 000 nucleotides). The Circular Consensus Sequence (CCS) software generates CCS reads, which involves capturing multiple subreads from the same SMRTbell™ molecule. These subreads are combined using a statistical model to produce a highly accurate consensus sequence, referred to as a HiFi read, with associated quality values. The CCS workflow within SMRT® Link uses this software tool for analysis.

Metagenomic analysis and taxonomic profiling

After evaluating the quality of raw reads, Trimmomatic version 0.32 was used to remove low-quality reads with a Q25 threshold. The filtered paired-end sequence data were merged using VSEARCH version 2.13.4 with default

parameters. Primers were trimmed using the alignment algorithm of Myers and Miller (1988), with a similarity cutoff of 0.8. Non-specific amplicons unrelated to the 16S rRNA gene were identified using nhmmer from HMMER software package version 3.2.1 with hmm profiles.

The next step involved extracting unique reads using VSEARCH2's "derep fulllength" command and clustering redundant reads with unique ones. Taxonomic assignment utilized VSEARCH2's usearch global command and the EzBioCloud 16S rRNA database, followed by more precise pairwise alignment. Chimeric reads were filtered using reference-based detection with the UCHIME algorithm and the non-chimeric 16S rRNA database from EzBioCloud with a 97% similarity threshold.

Unidentified reads in the EzBioCloud database below the species level were combined. The "cluster_fast" program in VSEARCH2 was used for de novo clustering, generating new Operational Taxonomic Units (OTUs). Single-read OTUs were excluded. Further analyses, including diversity calculation and biomarker identification, employed in-house algorithms by Chunlab, Inc.

Various alpha diversity indices, including ACE, Chao1, Jackknife, Shannon, NPShannon, Simpson and Phylogenetic diversity, were calculated. Rarefaction and rank abundance curves were assessed. All analyses were performed in EzBioCloud's 16S-based MTP using the PKSSU 4.0 database.

Screening of lipase-producing bacteria from consortium BS14

The presence of lipolytic bacteria in the consortium was screened through qualitative (rhodamine-olive-oil, or ROA, and Tween 80 plate assay), and quantitative spectrophotometric assay. For these two tests, bacterial consortium cultures were grown in statistically optimised media that were supplemented with WCO (0.5% v/v) or PCO (1.25% v/v), and were spread onto ROA agar plates (0.8% NB, 0.4% (w/v) NaCl, 500 µL 0.01% rhodamine B solution, 1% (w/v) bacteriological agar and 7.5% (v/v) olive oil), pH 7 (Rabbani *et al.* 2013). The concentration of WCO and PCO was selected from the past study on the optimisation of biodegradation of WCO and PCO using same bacterial consortium (Zahri *et al.* 2021). Based on this paper, the degradation process took 6 days. The plates were incubated at 13°C for the same duration, and were then exposed to UV light at 365 nm to determine the lipase activity of the isolates. Colonies showing fluorescence were selected for further screening (Alhamdani and Alkabbi 2016).

Secondary screening was carried out using Tween 80 agar plates to confirm lipolytic activity. Plates containing the oil substrate were prepared as follows: 10 g L^{-1} peptone, 1.3 g L^{-1} NaCl. 0.1 g L^{-1} $CaCl_2 \cdot 2H_2O$, 20 g L^{-1} bacteriological agar and 10 ml L^{-1} of Tween 80 (Alhamdani and Alkabbi 2016). The positive bacterial colonies from the ROA plates were subcultured into fresh NB and grown for 3 days before being used to inoculate the Tween 80 plates, which were incubated at 10°C for 6 days. This process served as an intermediate step, allowing the bacteria to grow and adapt before being transferred to the Tween 80 plates.

Characterisation and identification of lipase-producing bacteria from consortium BS14 using Sanger sequencing of the 16S rRNA gene

The first identification step for the isolated bacteria was based on morphological observation, where the bacteria grown on Tween 80 agar plates were identified through Gram staining. More definitive identification was then performed using 16S rRNA sequencing to assist in bacterial classification.

Genomic DNA extraction was performed using the Macherey-Nagel bacterial extraction kit following the manufacturer's instructions. To amplify the 16S rRNA gene, PCR was carried out using forward primer 27F and reverse primer 1492R at a concentration of 10 µM (Schulze-Schweifing *et al.* 2014). The PCR reaction had a total volume of 25 µL, consisting of 12.5 µL 2X *Taq* Master Mix (Vivantis, USA), 1 µL of each primer, 9.5 µL distilled water and 1 µL genomic DNA.

The PCR reaction was performed using a Bio-Rad Thermal Cycler under the following conditions: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53.5°C for 1 min and extension at 72°C for 2 min, and a final extension at 72°C for 10 min. The reaction was then held at 4°C. The PCR products were analyzed by running them on an agarose gel using a VC 1kb-Ex DNA ladder (Vivantis, USA) as size standard.

Following gel electrophoresis, the PCR products were purified using the QIAquick PCR purification kit (Qiagen, Germany). The purified PCR products were sent to Next-Gene Scientific Sdn. Bhd. (Selangor, Malaysia) for 16S rRNA sequencing. The obtained sequences were compared with sequences deposited in the GenBank database using the BLAST program provided by the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov.

The obtained sequences were aligned for similarity using the standard NCBI database (reference RNA sequences) using the nucleotide basic local alignment search tool (BLASTn). The ClustalW programme generated multiple alignments of the nucleotide gene sequences. Subsequently, a phylogenetic tree with the best substitution model tested with 1 000 bootstrap values for tree evaluation was constructed using MEGAX software version 10.2 (Alsultan *et al.* 2019). The best DNA models were tested first before constructing the phylogenetic tree. Models tested include HKY, K2, general time-reversible (GTR), Tamura-Nei (TN93), Tamura-3-parameter (T92) and Jukes-Cantor (JC).

The bootstrap percentage values shown at the internal nodes of the phylogenetic tree were based on 1 000 iterations. Bootstrapping was used to estimate the confidence

level of the branches in the tree. A bootstrap value above 70% is considered well-supported, indicating the repeatability of the data and the probability of retrieving the same clade using an independent dataset (Lemoine *et al.* 2018). Clades produced in less than 50% of the bootstrap replicates were collapsed, as described by Russo and Selvatti (2018).

Results

Extraction of genomic DNA

The presence of genomic DNA from all samples (OC, WT and PT) tested was confirmed using the Macherey-Nagel extraction kit on agarose gel electrophoresis (AGE) (Fig. 1). The integrity of the genomic DNA was evidenced using AGE, where intact DNA with high molecular mass (> 10 000 bp) was observed in all samples.

The concentration and purity of the obtained genomic DNA were assessed spectrophotometrically by determining the absorbance ratios at A260/A280 and A260/A230. Table 1 presents the DNA yields and concentration, as well as purity values for all samples. The average ratio of aforementioned absorbance was > 1.8, indicating that the genomic DNA obtained was of high quality. The level of sample contamination was evaluated based on the absorbance readings at 260 nm and 230 nm, where ratios below 1.8 would suggest the presence of significant contamination (Shen 2019). The results obtained further confirm that high-quality genomic DNA was successfully extracted from all samples. Consequently, these DNA samples were subjected to subsequent analyses.

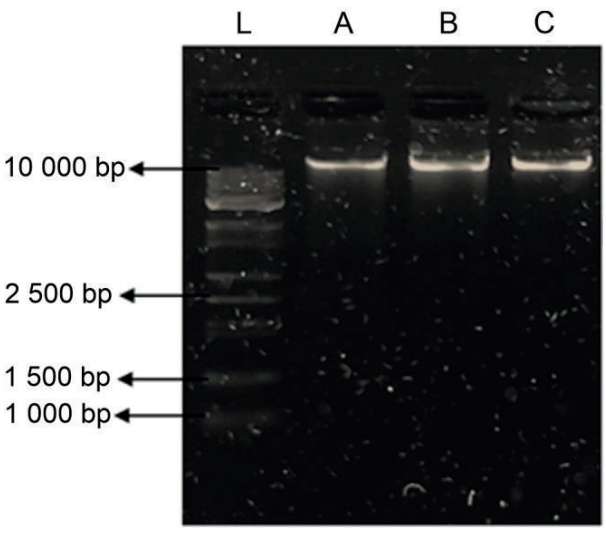

Fig. 1. Agarose gel electrophoresis of genomic DNA extracted from bacterial consortium from A) OC, B) WT, C) PT. L: VC 1kb-Ex DNA ladder.

Full-length 16S rRNA genes were amplified from the bacterial communities derived from the BS14 consortium after exposure to three different treatment conditions. Table 2 summarizes the PacBio reads obtained for each sample, including polymerase reads and subreads. Each polymerase read comprises one or more subreads, which contain sequences from a single polymerase pass on a single strand of an insert within a SMRTbell™ template, excluding adapter sequences. The average length of the polymerase reads across all three samples was 4 270 984 910 with a mean size of 33 871 nucleotides (nt). Raw reads were initially assembled, demultiplexed and tagged with identical barcodes located at both ends of an insert. Circular Consensus Sequence (CCS) reads were then generated. The number of CCS reads with full passes varied across the samples, with 79 755 reads for OC, 94 802 reads for WT and 76 329 reads for PT. Chimeric and CCS reads shorter than 1 000 nt were removed. The resulting validated full-length 16S reads ranged from 66 940 to 86 117 reads per sample, with an average read length of 1 503 base pairs.

In this study, the number of Operational Taxonomic Units (OTUs) achieved per sample at a 97% similarity level ranged from 40 to 69, indicating variation in bacterial OTU richness across the different culture treatment conditions (Table 2). Based on the various non-parametric indices including Chao1, ACE, Jackknife, Shannon index, Simpson, and NPShannon, which assess species richness and evenness of the microbial consortium, the non-treated sample (OC) exhibited the highest values (Burnham and Overton 1979; Chao 1987; Chao and Lee 1992; Magurran 2013). The diversity indices Chao1, ACE and Jackknife showed that species richness decreased in the following order: OC > PT > WT. Similarly, the Shannon, Simpson and NPShannon indices yielded comparable results for species evenness and diversity. Higher values of Shannon and NPShannon indicate greater diversity, while lower values of Simpson suggest higher diversity. The phylogenetic diversity index, which assesses biodiversity of the bacterial community, revealed that the OC sample had low phylogenetic diversity (40.0) compared to the bacterial consortium from WT (69.0). Therefore, the OC sample exhibited the highest species richness and evenness but low phylogenetic diversity.

Rarefaction curves (Fig. 2) further support the observation that greater bacterial diversity was present com-

Table 1. Yields of genomic DNA for original culture and canola oil-treated samples.

Sample	DNA concentration (ng μL^{-1})	A_{260}/A_{280}	A_{260}/A_{230}
OC	566.5	1.94	2.03
WT	247.4	1.93	1.82
PT	322.2	1.92	1.94

Table 2. Summary of PacBio sequencing and statistical analysis.

Sequencing results		Sample		
		OC	WT	PT
Polymerase reads (bp)		4 235 347 823	4 617 266 921	3 960 339 986
Subreads (bp)		3 963 115 825	4 617 266 921	3 701 378 418
CCS analysis				
	Total input reads (bp)	120 149	140 402	114 289
	Below predicted accuracy (bp)	11 982	13 266	11 175
	Success CCS (%)	66.38	67.52	66.79
Number of total reads after pre-filter (bp)		71 075	87 606	68 567
Number of validated reads (bp)		67 688	86 117	66 940
Mean read length (bp)		1 501	1 504	1 505
Maximum read length (bp)		73 278	77 802	99 757
Number of operational taxonomic units (OTUs)		69	40	44
Good's coverage (%)		100	100	100
	Chao1	69.5	40.9	45.1
	ACE	71.2	42.5	47.6
	Jackknife	75.0	46.0	51.0
	Shannon	0.713	0.082	0.188
	Simpson	0.507	0.976	0.936
	NPShannon	0.715	0.083	0.190
	Phylogenetic	40.0	69.0	41.0

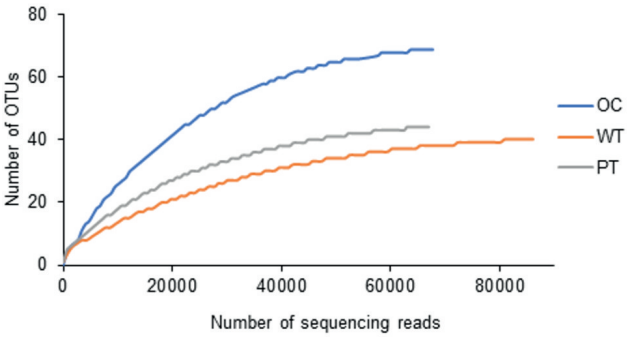

Fig. 2. Rarefaction curves for numbers of operational taxonomic units (OTUs) assigned to the bacterial consortium from the original culture (OC) and after treatment with WCO (WT) or PCO (PT), using a 97% similarity of cut-off value. The termination point of the curves signifies the number of sequences.

pared to WT and PT. The diversity indices presented in Table 2 also align with the species richness results for OC.

Bacterial community composition in non-treated conditions

The diversity and composition of the identified bacterial community clearly differed between the OC conditions and the treatment conditions (WT and PT). The Microbiome Taxonomic Profile (MTP) sequences of the bacterial 16S rRNA gene were classified from the phylum to the genus level. For OC, the taxonomic analysis revealed association with three phyla, four classes, four orders, six families and seven genera. As shown in Fig. 3A, by far the predominant phyla were Proteobacteria (57.03%) and Firmicutes (42.96%), accounting for 99.99% of the sequences assigned. In contrast, the bacterial community after WT was dominated by members of Proteobacteria (99.12%), with a much smaller proportion of Firmicutes (0.87%). Very low percentages of other phyla were also detected in WT, including 0.0015% Bacteriodetes, 0.0093% Actinobacteria, 0.0035% Planctomycetes, 0.0012% Cyanobacteria and 0.0012% Verrucomicrobia. These phyla and Firmicutes were considered to be present in minor quantities, comprising a total of only 0.88%. These were grouped under the category of ETC with the threshold for ETC classification being set at 1.0% The WT community included eight classes, 11 orders, 12 families and 18 genera. The bacterial community in PT was also dominated by Proteobacteria (97.46%), with a lower contribution of Firmicutes (2.54%). The PT OTU diversity included somewhat lower numbers of four classes, four orders, six families and six genera.

At the family level, the bacterial community from OC was primarily dominated by Pseudomonadaceae (57.03%) and Carnobacteriaceae (42.96%) (Fig. 3B). Other families, including Moraxellaceae, Flavobacteriaceae, Neisseriaceae

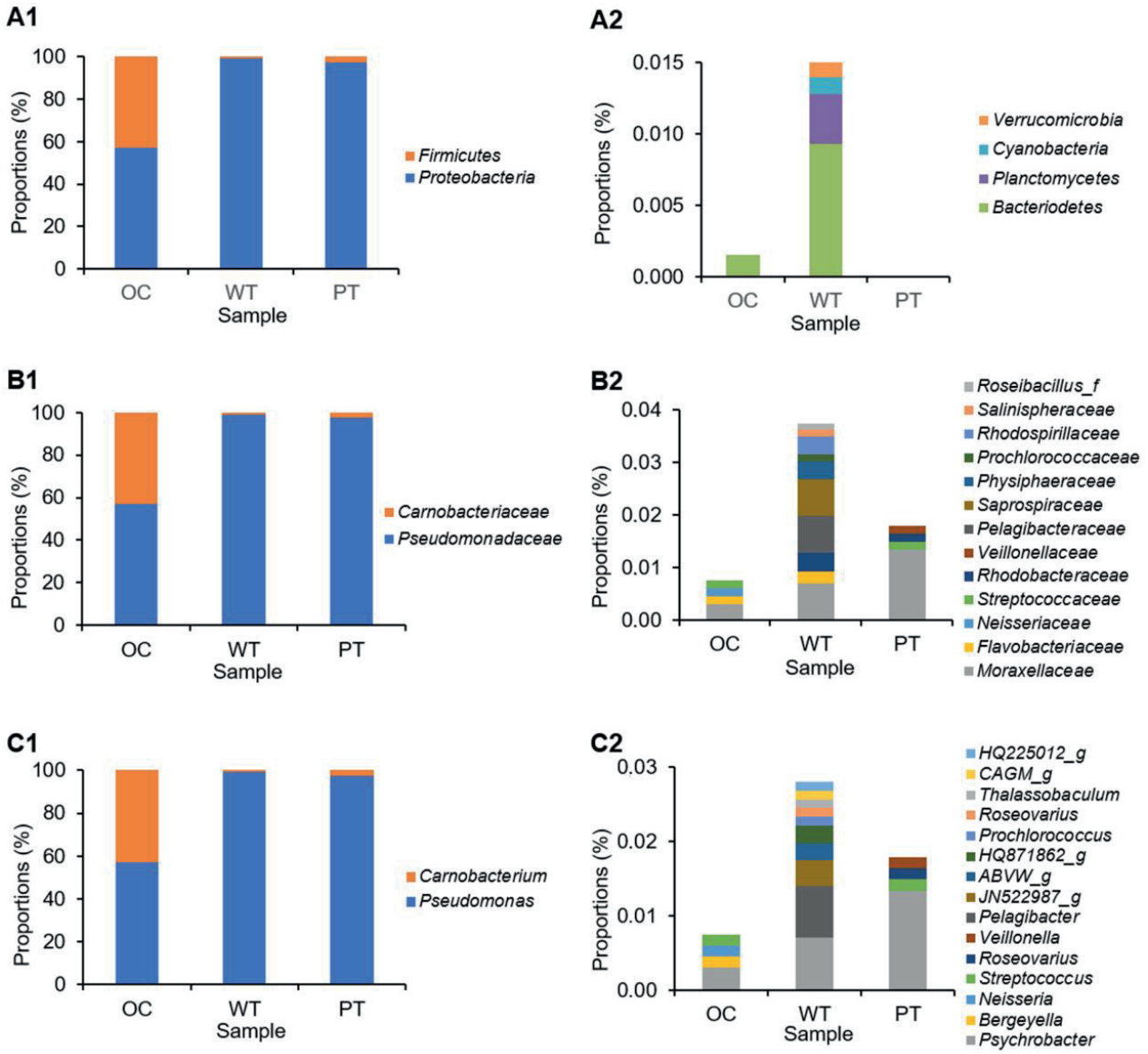

Fig. 3. Taxonomic classification at (**A**) phylum, (**B**) family and (**C**) genus level of bacterial communities (OTU diversity) present under the three treatment conditions of the BS14 consortium, (**1**) major and (**2**) minor components of the bacterial community.

and Streptococcaceae, were present in smaller proportions ranging from 0.0030% to 0.0015%. In both WT and PT, Pseudomonadaceae became the dominant family, accounting for 99.10% in WT and 97.45% in PT, while Carnobacteriaceae were reduced to 0.877% in WT and 2.53% in PT. Several additional families were identified in WT. Rhodobacteraceae, Pelagibacteraceae, Saprospiraceae, Rhodospirillaceae, Salinisphaeraceae and Roseibacillus_f were present in low proportions ranging from 0.0012% to 0.007%. The representation of other bacterial families was 0.01%, 0.9% and 0.02% for OC, WT and PT, respectively.

At the genus level, the community of OC was dominated by *Pseudomonas* (57.02%) and *Carnobacterium* (42.96%), representing the orders Pseudomonadales and Lactobacillales, respectively. Minority genera included *Psychrobacter* (0.003%), *Bergeyella* (0.0015%), *Neisseria* (0.0015%), *Streptococcus* (0.0015%) and others (0.01%). In WT, the dominant genus was *Pseudomonas* (99.10%), with much lower presence of *Carnobacterium* (0.87%), *Psychrobacter* (0.007%), *Pelagibacter* (0.007%), *JN52 2987_g* (0.0035%), *ABWW_g* (0.0023%), *HQ871862_g* (0.0023%), *Prochlorococcus* (0.0012%), *Resovarius* (0.0012%), *Thalassobaculum* (0.0012%), *CAGM_g* (0.0012%), *HQ225012_g* (0.0012%) and others (0.9%) (Fig. 3C). Similarly, in PT, *Pseudomonas* was dominant (97.45%), followed by *Carnobacterium* (2.53%), *Psychrobacter* (0.013%), *Streptococcus* (0.0015%), *Roseovarius* (0.0015%), *Veilonella* (0.0015%) and others (0.02%).

Population-specific OTU diversity at species level

Table 3 summarises the abundance of assigned OTUs at the species level. Members of the *Pseudomonas fluorescens* group and *Carnobacterium maltaromaticum* had high relative abundances of 84.12% and 15.4%, respectively,

Table 3. Relative abundances of bacteria assigned at the species level present in the communities under OC, WT and PT treatments.

Taxonomy			Relative abundance (%)	Sample		
Class	Order	Genus/species		OC	WT	PT
Alphaproteo-bacteria	Pelagibacterales	*Pelagibacter KL370779_s* group	0.007			
	Rhodobacterales	*HQ871862_g HQ871862_s*	0.002			
		Roseovarius nubinhibens	0.001			
		Roseovarius aestuariivivens	0.002			
	Rhodospirillales	*Thalassobaculum AY258092_s*	0.001			
Betaproteobacteria	Neisseriales	*Neisseris subflava*	0.002			
Gammaproteo-bacteria	Pseudomonadales	*Pseudomonas fluorescens* group	84.12			
		Pseudomonas synxantha group	0.29			
		Pseudomonas lini group	0.008			
		Pseudomonas_uc	0.098			
		Pseudomonas amygdali group	0.004			
		Pseudomonas fragi group	0.002			
		Pseudomonas chlororaphis group	0.002			
		Pseudomonas cedrina group	0.002			
		Pseudomonas frederiksbegensis	0.002			
		Psychrobacter cryohalolentis	0.007			
		Psychrobacter okhotskensis group	0.003			
		Psychrobacter proteolyticus group	0.001			
		Psychrobacter glacincola	0.002			
	Salinisphaerales	*HQ225012_g HQ225012_s*	0.002			
Bacilli	Lactobacillales	*Carnobacterium maltaromaticum*	15.14			
		Carnobacterium divergens group	0.26			
		Carnobacterium_uc	0.054			
		Carnobacterium funditum group	0.002			
		Streptococcus salivarius group	0.002			
		Streptococcus pneumoniae group	0.002			
Flavobacteria	Flavobacteriales	*ABVW_g ABVW_s*	0.002			
		PAC001338_s	0.002			
Verrucomicrobiae	Verruomicobiales	*CAGM_g GQ850568_s*	0.001			
Chroobacteria	Chroococcales	*Prochlorococcus LT578417_s*	0.001			
Negativicutes	Veilonellales	*Veillonella rogosae*	0.002			
		ETC (< 0.1%)	0.997			
		Unclassified	0.151			

Abundance:						
	0%	< 0.001%	< 0.1%	< 1%	< 10%	10–100%

dominating the bacterial communities in all three treatments. Other species assigned in most treatments included the *Pseudomonas synxantha* group, *Pseudomonas_uc*, *Carnobacterium divergens* group and *Carnobacterium_uc*.

The WT treatment generated the highest number of sequencing reads at species level (86 050), with OC and PT treatments generating 67 511 and 66 851 reads, respectively. Species representing Alphaproteobacteria were mainly detected in the WT treatment. The *Psychrobacter proteolyticus* group, *HQ225012_g*, *HQ225012_s*, *PAC001338_s* and *CAGM_g GQ850568_s*, were also identified at the species level, but their relative abundances were very low.

The relative abundance of the six most abundant taxa at the species level increased in the following order: *Carnobacterium_uc* < *Pseudomonas_uc* < *Carnobacterium divergens* group < *Pseudomonas synxantha* group < *Carnobacterium maltaromaticum* < *Pseudomonas fluorescens* group (Table 4). The highest relative abundance within the *Pseudomonas fluorescens* group was observed in the WT treatment (98.77%), whilst the lowest abundance was found in the OC treatment (56.88%). Conversely, *Carnobacterium maltaromaticum* had the lowest species abundance in the WT treatment (0.79%), followed by PT (1.82%) and OC (42.82%). The *Pseudomonas synxantha* and *Carnobacterium divergens* groups exhibited similar relative abundances in each of the canola oil treatments. These two taxa were not detected in the OC treatment, while their abundances in the WT and PT treatments showed ranges of 0.08–0.25% and 0.62–0.69%, respectively. The relative abundance of *Pseudomonas_uc* was 0.12% in the OC treatment, 0.06% in WT and 0.11% in PT. Finally, the relative abundance of *Carnobacterium_uc* was 0.13% in OC, not detected in WT and 0.02% in PT.

The most similar bacterial 16S rRNA sequences available in the GenBank database to those obtained in the current study are presented in Table 5. However, it is important to note that the 16S profiling approach used in this

Table 4. Relative abundance of the six most commonly assigned operational taxonomic units across all three treatments of the BS14 consortium.

Species	Relative abundances (%)		
	WT	PT	OC
Pseudomonas fluorescens group	98.77	96.7	56.88
Carnobacterium maltaromaticum	0.79	1.82	42.82
Pseudomonas synxantha group	0.25	0.62	ND
Carnobacterium divergens group	0.08	0.69	ND
Pseudomonas_uc	0.06	0.11	0.12
Carnobacterium_uc	ND	0.02	0.13

Table 5. List of bacterial species from the identified species groups (relative abundance > 0.1%) from non-treated and canola oil-treated samples with close 16S rRNA sequence similarity to taxa included in the GenBank database.

Phylum (class)	Top hit of species group (>0.1%)	Species	Strain	Similarity (%)
Proteobacteria (gammaproteobacteria)	*Pseudomonas fluorescens* group	*Pseudomonas fluorescens*	DSM 50090	99.3
		Pseudomonas marginalis	ATCC 10844	99.2
		Pseudomonas veronii	DSM 11331	99.7
		Pseudomonas rhodesiae	CIP 104664	99.4
		Pseudomonas orientalis	CFML 96-170	99.3
		Pseudomonas costantinii	CFBP 5705	99.2
		Pseudomonas extremorientalis	KMM 3447	99.4
		Pseudomonas grimontii	CFML 97-514	99.5
		Pseudomonas trivalis	DSM 14937	99.4
		Pseudomonas poea	DSM 14936	99.4
		Pseudomonas meridiana	CMS 38	99.4
		Pseudomonas antarctica	CMS 35	99.5
		Pseudomonas simiae	OLi	99.5

Table 5 continued

Phylum (class)	Top hit of species group (>0.1%)	Species	Strain	Similarity (%)
		Pseudomonas lurida	LMG 21995	99.2
		Pseusdomonas extremaustralis	14-3	99.7
		Pseudomonas canadensis	2-92	99.2
	Pseudomonas synxantha group	*Pseudomonas synxantha*	DSM 18928	99.1
		Pseudomonas mucidoiens	LMG 2223	99.0
		Pseudomonas azotoformans	DSM 18862	98.9
		Pseudomonas libanensis	CIP 105460	99.0
		Pseudomonas gessardii	DSM 17152	99.2
		Pseudomonas brenneri	CFML 97-391	99.3
		Pseudomonas proteolytica	CMS 64	99.0
		Pseudomonas panacis	CG20106	98.8
		Pseudomonas yamanorum	8H1	99.3
		Pseudomonas paralactis	DSM 29164	98.9
		Pseudomonas paralactis	DSM 29164	98.9
Firmicutes (Bacilli)	*Carnobacterium divergens* group	*Carnobacterium divergens*	DSM 20623	98.2
		FLLU_s	CDIV41	97.9

study may not provide reliable species-level resolution (Earl *et al.* 2018). This is due to the amplification and sequencing of only short 16S rRNA gene regions, typically providing for only family or genus taxonomy level.

Determination of lipase-producing bacteria within bacterial consortium BS14

Pure cultures of lipase-producing bacteria were successfully isolated from RSM optimised liquid medium on the last day of the degradation process. The cultures were transferred to ROA media plates for identification of bacteria responsible for the biodegradation of canola oil. Totals of seven and 10 isolates from MSM in WCO and PCO treatments, respectively, were positive from the ROA selective media plates. The fluorescence of the bacterial colonies was detected after 6 days of incubation. This indicated hydrolysis of the oil had taken place, with higher colour intensity indicating the higher lipid breakdown by the bacterial isolates. The results showed distinct precipitation zones, where isolates W7, P2 and P4 exhibited higher hydrolysis activity when Tween 80 was used as the substrate compared to that observed with olive oil in ROA plates (Table 6).

All isolates were classified as Gram-negative (pink/red) rods, except for one isolate (P3), which was identified as Gram-positive (Table 7). Additionally, the arrangement of several bacterial isolates varied, including bacillus, diplobacillus, streptobacillus and palisades forms. The isolated colonies were also evaluated based on pigmentation, optical density, margin, elevation and consistency. Most exhibited very similar characteristics, making accurate identification challenging. Molecular identification was therefore conducted.

Table 6. Best substitution model Lipase activity of bacterial isolates after exposure of consortium BS14 to WCO or PCO, assessed using Rhodamine B or Tween 80 plate assays.

Isolate	Rhodamine B	Tween 80
WCO		
W1	+++	++
W2	++	+++
W3	+	+
W4	++	+
W5	+++	++
W6	+++	++
W7	+	+++
PCO		
P1	++	++
P2	+	+++
P3	+	++
P4	+	+++
P5	+++	+
P6	+	++
P7	++	+++
P8	++	+
P9	++	+
P10	++	+
High activity (+++), moderate activity (++), low activity (+)		

Table 7. Characterisation of bacterial isolates from BS14 Antarctic soil bacterial consortium.

Isolate	Cell characteristics			Colony characteristics				
	Gram	Shape	Pigmentation	Optical density	Margin	Elevation	Consistency	Arrangement
WCO								
W1	-ve	Rod	Yellow	Translucent	Entire	Convex	Mucoid	Palisades
W2	-ve	Rod	Yellow	Opaque	Entire	Convex	Mucoid	Palisades
W3	-ve	Rod	Light yellow	Translucent	Entire	Convex	Mucoid	Bacillus
W4	-ve	Rod	Light yellow	Translucent	Entire	Convex	Buttery	Diplobacillus
W5	-ve	Rod	Light yellow	Translucent	Entire	Convex	Buttery	Diplobacillus
W6	-ve	Rod	Yellow	Opaque	Entire	Convex	Buttery	Diplobacillus
W7	-ve	Rod	Yellow	Opaque	Entire	Convex	Buttery	Diplobacillus
PCO								
P1	-ve	Rod	Light yellow	Translucent	Entire	Convex	Mucoid	Streptobacillus
P2	-ve	Rod	Yellow	Opaque	Entire	Convex	Mucoid	Streptobacillus
P3	+ve	Rod	Light yellow	Translucent	Entire	Convex	Mucoid	Diplobacillus
P4	-ve	Rod	Yellow	Translucent	Entire	Convex	Mucoid	Bacillus
P5	-ve	Rod	Yellow	Opaque	Entire	Convex	Mucoid	Diplobacillus
P6	-ve	Rod	Yellow	Opaque	Entire	Convex	Buttery	Bacillus
P7	-ve	Rod	Light yellow	Translucent	Entire	Convex	Buttery	Diplobacillus
P8	-ve	Rod	Light yellow	Translucent	Entire	Convex	Buttery	Bacillus
P9	-ve	Rod	Yellow	Translucent	Entire	Convex	Buttery	Palisades
P10	-ve	Rod	Yellow	Translucent	Entire	Convex	Buttery	Palisades

Extraction and amplification of 16S rRNA gene for molecular identification of isolated bacteria

The genomic DNA of the lipase-producing bacterial isolates was successfully extracted. A single band was formed for each of the WCO isolates on the 1% agarose gel, and additional faint bands were present in several PCO isolates. All isolates had PCR-amplified fragments of around 1 500 bp, and the presence of single intact and clear bands on the gel was successfully confirmed (Fig. 4).

Phylogenetic tree analysis

The results showed that Hasegawa-Kishino-Yano (HKY) and Kimura 2 parameters (K2) models with gamma and invariable evolutionary rates were the most suitable models for constructing the phylogenetic tree of the isolated samples (Table 8). The phylogenetic analysis revealed that all isolates from media supplemented with WCO represented the genus *Pseudomonas*. Among the PCO isolates, most also represented *Pseudomonas*, except for one representing *Carnobacterium* (Fig. 5). Isolates W1-W7, P2, P4, P5, and P7-P10 clustered together with the type strains of *P. antarctica* strain CMS 35T and *P. meridiana* strain CMS 38T. P1 and P6 clustered with *P. versuta*, while isolate P3 clustered together with *Carnobacterium maltaromaticum*. The sequences from each isolate were used to construct individual molecular phylogenies for identification purposes.

As noted above, isolates *Pseudomonas* sp. strain AQ5-16 (W5), *Pseudomonas* sp. strain AQ5-18 (W7) and *Pseudomonas* sp. strain AQ5-19 (P1) had low values of percentage identity (Table 9), suggesting they may represent distinct species or genera. Although isolates *Pseudomonas* sp. strain AQ5-13 (W2), strain AQ5-14 (W3), strain AQ5-15 (W4), strain AQ5-20 (P2), strain AQ5-22 (P4), strain AQ5-23 (P5), strain AQ5-26 (P8), strain AQ5-28 (P10) and *Carnobacterium maltaromaticum* strain AQ5-21 (P3) had high similarity values (≥ 97%), these samples did not share the same clade at the internal nodes to the closest taxa clustered together in the phylogenetic tree. Thus, confidence in assigning these isolates is limited to the genus level. Assignments of all 17 isolates are given in Table 9, together with their GenBank accession numbers.

Discussion

Bioremediation of hydrocarbons in Antarctica has generated significant interest in recent years (Lim *et al.* 2021). The use of microorganisms to reduce pollution through the biological degradation of pollutants into less toxic or non-toxic products has been widely implemented, especially for the treatment of fuels and other types of oils (Salari

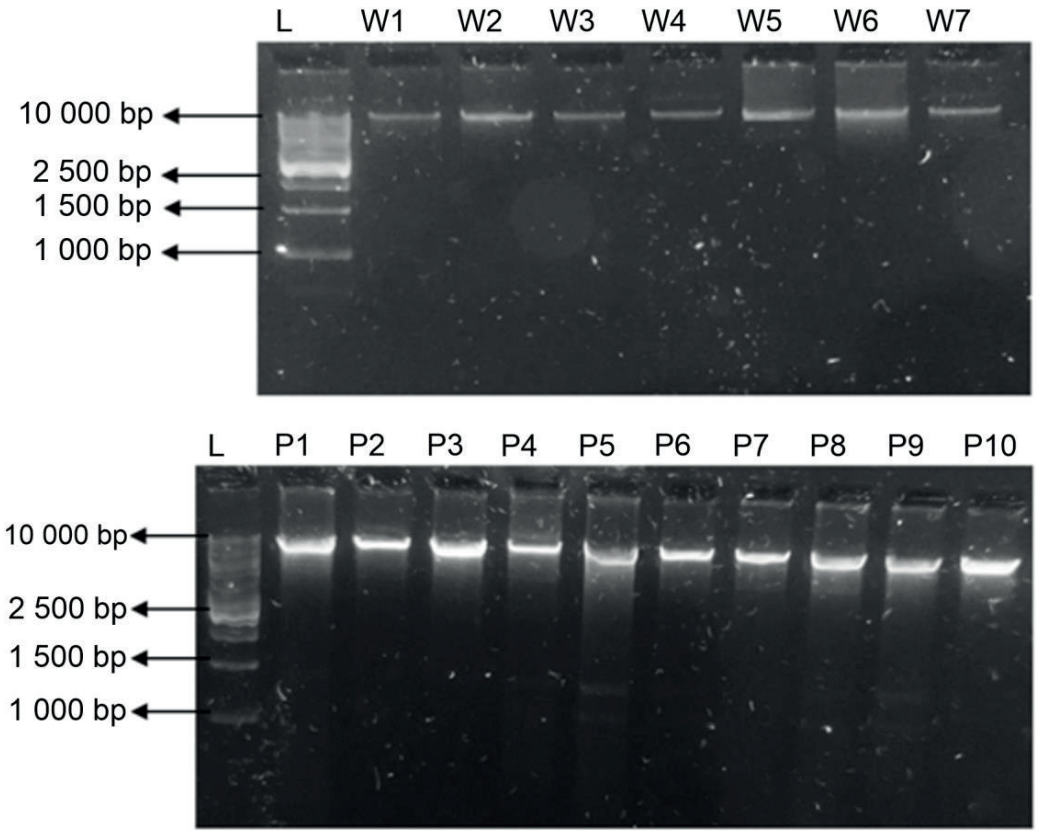

Fig. 4. Agarose gel electrophoresis of genomic DNA extracted from bacteria isolated in treated media supplemented with WCO (W) and PCO (P). L: VC 1kb-Ex DNA ladder (Vivantis, USA).

Table 8. Best substitution model tested for construction of phylogenetic tree for 20 sequences in each sample.

Sample	Model	BIC	AICc
W1	HKY + G	7270.951	6922.499
W2	HKY + G	7314.733	6966.291
W3	HKY + G	7176.909	6865.658
W4	HKY + G	7278.605	6930.198
W5	HKY + G	7330.768	6982.289
W6	HKY + G	7159.361	6829.479
W7	HKY + G	8093.146	7706.977
P1	HKY + G	7550.946	7239.408
P2	HKY + G	7166.782	6818.350
P3	K2 + G + I	9676.283	9381.515
P4	HKY + G	7096.128	6766.265
P5	K2 + G + I	13910.958	11908.906
P6	K2 + G + I	7912.852	7581.152
P7	K2 + G	7304.283	6980.686
P8	HKY + G	7439.629	7072.471
P9	K2 + G	7446.099	7123.218
P10	HKY + G	7250.997	6902.563

et al. 2022). To ensure environmental protection during the disposal of waste cooking oil stored in oil waste containers in Antarctica, it is essential to adopt appropriate disposal methods, given the substantial production of waste cooking oil in this region.

Bacterial consortia from Antarctica have been recognized for their ability to degrade waste cooking oil, particularly canola vegetable oil (Zahri *et al.* 2021). Metagenomic analysis enables the identification of canola oil-degrading bacteria during the bioremediation processes. Prior to microbiome taxonomic profiling, several stringent criteria were applied to filter out low-quality sequence reads. The stochastic errors associated with single-pass sequences are mitigated with each Circular Consensus Sequence (CCS) pass, as CCS enables repeated sequencing of specific inserts. Since PacBio raw reads do not have systematic errors, consensus insert sequences generated from multi-pass reads can achieve an average accuracy of over 99%.

The slope of the rarefaction curve can provide insights into the predicted species diversity, with initial rapid growth indicating the discovery of common species and subsequent flattening representing the detection of rarer species (CD Genomics 2022). A study of bacterial diversity in soil aliquot samples from Admiralty Bay, King George Island, similarly reported Proteobacteria and Firmicutes as the most abundant phyla in all tested samples (Teixeira *et al.* 2010).

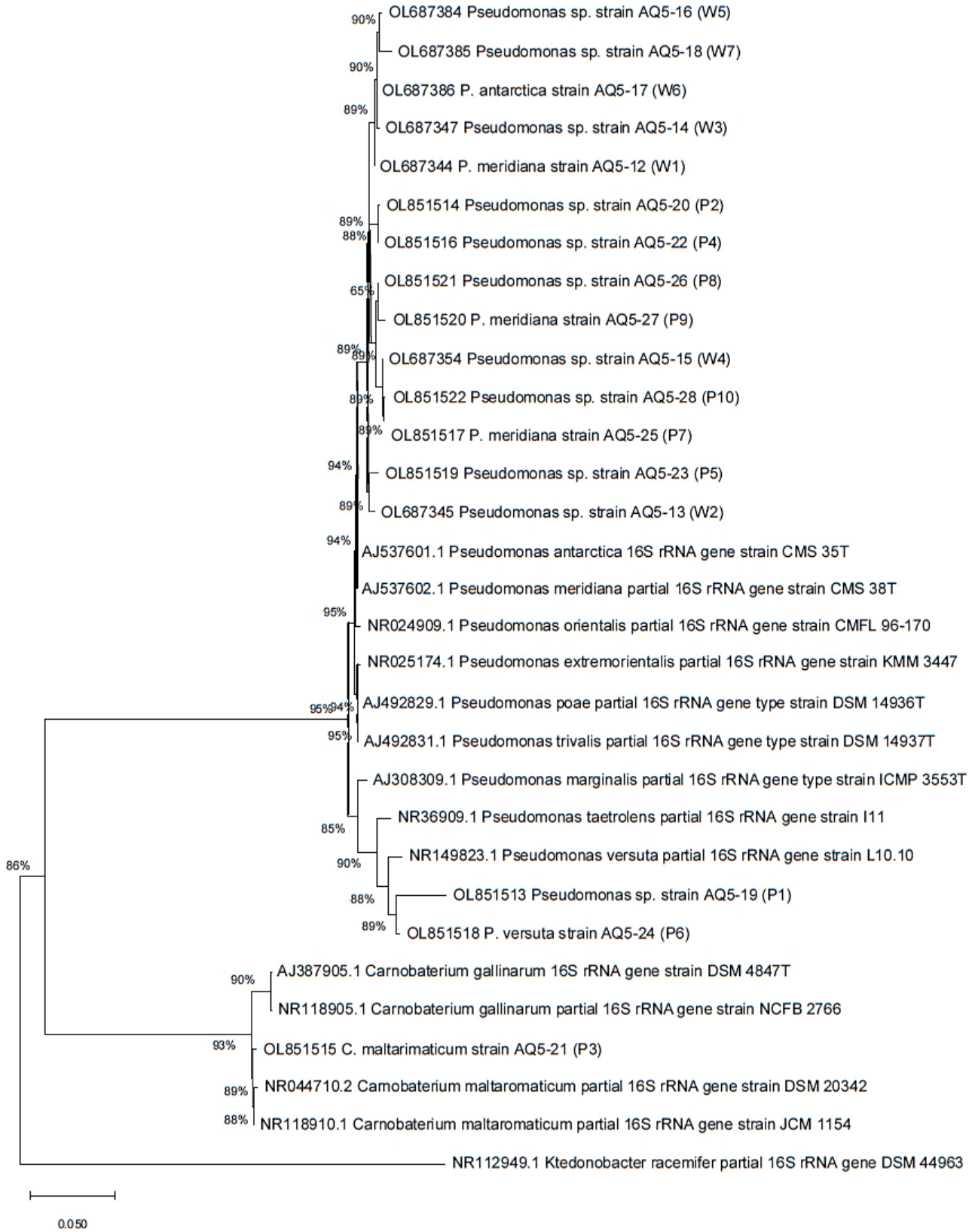

Fig. 5. Phylogenetic tree including 17 Antarctic lipase-producing bacterial isolates constructed using the Neighbor Joining method based on 16S rRNA gene sequences. Bootstrap value (%) from 1 000 iterations are shown at the branch nodes, indicating the robustness of each clade. The scale bar corresponds a 5% nucleotide sequence divergence. Well-characterized reference sequences from *Pseudomonas* and *Carnobacterium* species were included for taxonomic comparison and obtained from the NCBI GenBank database. The tree highlights the genetic diversity among the isolates and their phylogenetic proximity to known lipase-producing taxa, suggesting potential for biotechnological applications in cold environments. *Ktedonobacter racemifer* was used as outgroup.

Table 9. Taxonomic assignments of the 17 lipase-producing isolates based on sequence similarities with the NCBI GenBank database.

Isolate	Closest phylogenetic relative	Identification	Strain names	Accession number
W1	*P. meridiana* CMS 38T	*P. meridiana* AQ5-12	AQ5-12	OL687344
W2	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-13	AQ5-13	OL687345
W3	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-14	AQ5-14	OL687347
W4	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-15	AQ5-15	OL687354
W5	*P. meridiana* CMS 38T	*Pseudomonas* sp. AQ5-16	AQ5-16	OL687384
W6	*P. antarctica* CMS 35T	*P. antarctica* AQ5-17	AQ5-17	OL687386
W7	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-18	AQ5-18	OL687385
P1	*P. versuta* L10.10	*Pseudomonas* sp. AQ5-19	AQ5-19	OL851513
P2	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-20	AQ5-20	OL851514
P3	*C. maltaromaticum* DSM 20342	*C. maltaromaticum* AQ5-21	AQ5-21	OL851515
P4	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-22	AQ5-22	OL851516
P5	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-23	AQ5-23	OL851519
P6	*P. versuta* L10.10	*P. versuta* AQ5-24	AQ5-24	OL851518
P7	*P. meridiana* CMS 38T	*P. meridiana* AQ5-25	AQ5-25	OL851517
P8	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-26	AQ5-26	OL851521
P9	*P. meridiana* CMS 38T	*P. meridiana* AQ5-27	AQ5-27	OL851520
P10	*P. antarctica* CMS 35T	*Pseudomonas* sp. AQ5-28	AQ5-28	OL851522

Disturbance is a key factor influencing species diversity and variations in ecosystem structure (Santillan *et al.* 2019). The intermediate disturbance hypothesis (IDH), in particular, predicts that diversity will peak at intermediate levels of disturbance due to trade-offs between species' ability to compete, colonize ecological niches and tolerate disturbance (Connell 1978). Community diversity is affected by both biotic interactions and abiotic factors, such as local environmental conditions (Ma *et al.* 2022). Disturbance can stimulate community development involving many phylogenetically distinct microorganisms. These diverse communities occupy a wide range of niches and consume a variety of substrates produced during the degradation of organic matter (Galand *et al.* 2016). This explanation may account for why the bacterial community in WT had higher species-level diversity than that in the initial OC in this study, despite the relative abundance being dominated by a single assigned taxon. In an analogous experimental study, different frequencies of disturbance through augmentation with a toxic pollutant affected bacterial community diversity and ecosystem function in a closed microcosm bioreactor system (Santillan *et al.* 2019).

The extreme environmental conditions of Antarctica do not preclude the presence of diverse bacterial populations in its soils. These bacterial communities are likely to be regionally adapted and vary both within and between different parts of the continent, influenced by specific soil properties and environmental factors (*e.g.*, Chong *et al.* 2012; Bottos *et al.* 2014; Chong *et al.* 2015). Bacterial species are the most commonly identified prokaryotes in Antarctica (Lambrechts *et al.* 2019). Members of the genus *Pseudomonas* are often highly abundant in Antarctic soils across the continent (*e.g.*, Vazquez *et al.* 2013; Muangchinda *et al.* 2014; González-Rocha *et al.* 2017). In addition to *Pseudomonas*, other genera commonly reported in Antarctic soils include *Flavobacterium*, *Arthrobacter*, *Carnobacterium*, *Clostridium*, *Psychrobacter* and *Paenibacillus* (Lambrechts *et al.* 2019). The high abundance of *Pseudomonas* and *Carnobacterium* in the bacterial community of OC in this study is consistent with previous studies. The current investigation aims to provide an updated understanding of the prokaryotic diversity in continental Antarctic soils, with particular emphasis on cultivatable groups. The representatives of *Pseudomonas* and *Carnobacterium* detected here are clearly cultivable meaning they are of interest due to their potential use in bioremediation processes as demonstrated in both the WT and PT treatments.

Soil is one of the most complex natural ecosystems. Its habitats experience sporadic disturbance events that can induce discrete changes in their physical or chemical environments, potentially impacting the soil microbial community (Glasby and Underwood 1996). Community responses may include stabilization, resistance, sensitivity, or resilience. The data obtained in this study indicate that the initial composition of consortium BS14 changed as a result of being exposed to WT and PT treatments. In particular, the already common genus *Pseudomonas* became dominant, at the expense of a large reduction in the abundance of the genus *Carnobacterium*. This response indicates sensitivity

to disturbance. Comparing 310 experimental and 68 observational studies of microbial responses to disturbance, Shade *et al.* (2012) concluded that 82% of studies reported sensitivity to disturbance, expressed as changes in community composition (26%), function (21%), or both (35%). The studies involved large-scale disturbances (such as changes in temperature, deforestation), small-scale disturbances (nutrient amendment, alteration of temperature, fumigation) and *in situ* investigations.

Such differences in enzyme activity between strains and substrates used have been reported in other studies. For instance, Carissimi *et al.* (2007) tested 17 strains of *Sporothrix schenckii* on ROA and the Tween 80 assay to assess the level of lipase activity, finding that a majority of strains showed different fluorescence intensities and sizes of halo zones depending on the assay used.

Phylogenetic trees constructed in this study to illustrate the relationships among closely related strains were utilizing the maximum likelihood method, recognized as the most robust tree-building approach (Russo and Selvatti 2018). Most of the isolated bacteria were identified as closely related to the genus *Pseudomonas*. Several bacterial strains closely related to *Pseudomonas* species, including CMS 35T and CMS 38T, have previously been identified from Antarctica by Reddy *et al.* (2004). These strains were assigned to two novel species, *P. antarctica* and *P. meridiana*, respectively. Reddy *et al.* (2004) reported that both aforementioned strains, isolated from the McMurdo Sound region of continental Antarctica, exhibited lipase activity and were capable of growth within a temperature range of 4–30°C (optimum 22°C).

Members of the genus *Pseudomonas*, to which most of the isolated bacteria were assigned, are known for their metabolic versatility and ability to produce various extracellular enzymes, including lipases, in response to changing nutrient conditions. Previous studies have demonstrated the lipolytic activity of *Pseudomonas* species. For example, *Pseudomonas* sp. strain LSK25 isolated from Signy Island exhibited maximum lipolytic activity at low temperatures, and other strains such as LSK14, CF1 and BP1 also displayed high lipase activity (Salwoom *et al.* 2019). Other *Pseudomonas* strains have been isolated from terrestrial oases in continental Antarctica that show positive lipase and catalase activity without requiring specific enrichment media (Shivaji *et al.* 1989). Various *Pseudomonas* strains have high substrate specificity for fatty acids, and their ability to degrade cooking oil has been demonstrated (Nzila *et al.* 2016; Shi *et al.* 2021). Similarly, *Carnobacterium* species have also shown the ability to hydrolyze triglycerides such as tributyrin (Papon and Talon 1988).

The data obtained in this study highlight the specific potential of *Pseudomonas* and *Carnobacterium* species in the bioremediation of lipid-rich pollutants. Their enzymatic activities, specifically lipase production, make them effective agents for degrading different types of oils and fats. Furthermore, as demonstrated here, these bacteria are present in native bacterial consortia in the Antarctic environment, while other studies have also documented the prevalence of *Pseudomonas, Micrococcus, Alcaligenes, Flavobacterium* and *Aeromonas* species in Antarctica's terrestrial microflora.

Conclusions

The studied consortium, BS14, was primarily composed of *Pseudomonas fluorescens* group and *Carnobacterium maltaromaticum*. Exposure to either used or fresh canola oil induced rapid shifts in overall microbial consortium composition, while maintaining the dominance of *Pseudomonas* spp., underscoring their potential role in canola oil biodegradation. Lipase-producing bacteria isolated from treated WCO and PCO samples were identified as *P. meridiana*, *Pseudomonas* sp., *P. antarctica*, *Carnobacterium* sp. and *P. versuta* – all taxa commonly associated with Antarctic soils, arranged according to the order shown in Table 9. This study establishes a foundation for the application of microbial consortia in the bioremediation of cooking oil-contaminated environments, particularly those impacted by canola oil, associated with Antarctic wastewater systems or cooking oil waste storage. Further functional metagenomic research is needed to explore the genetic potential, evolutionary adaptations and ecological interactions of the involved microbial communities.

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