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Cold-adapted yeasts for hydrocarbon degradation: bioremediation insights from extreme environments

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ABSTRACT

Cold-adapted yeast strains were isolated from Continental Antarctic soils through hydrocarbon-based enrichment and characterized for their potential in low-temperature hydrocarbon degradation. Molecular identification revealed the isolates as *Rhodosporidiobolus odoratus*, *Rhodotorula mucilaginosa*, and *Filobasidium magnum*. Growth assays on 30 carbon (C) and 5 nitrogen (N) sources at 10 °C revealed differential metabolic traits among the strains, with *F. magnum* exhibiting the broadest substrate assimilation profile, including hexadecane utilization. Gas chromatography–mass spectrometry (GC-MS) was employed to evaluate the hydrocarbon-degrading capacity of the isolates in Bushnell-Haas broth supplemented with spent engine oil. The initial composition of the oil was dominated by C_{20}–C_{50} alkanes (78%), with minor fractions of methyl esters (11%), polycyclic aromatic hydrocarbons (PAHs; 5%), BTEX compounds (3%), C_1–C_{20} alkanes (2%), and alkylbenzenes (1%). After 30 days of incubation at 10 °C, significant differences in degradation efficiency were observed among the strains. *R. odoratus* significantly reduced long-chain alkanes, whereas both *R. odoratus* and *F. magnum* decreased PAHs and aromatic derivatives. *F. magnum* achieved the lowest residual relative abundance of BTEX and alkyl biphenyls. In contrast, *R. mucilaginosa* showed limited degradation capacity and in some cases, an accumulation of aromatic intermediates. These findings indicate that the yeast isolates *R. odoratus* and *F. magnum* exhibit promising hydrocarbon-degrading activity across multiple compound classes, including both aliphatic and aromatic hydrocarbons. Hydrocarbon degradation by cold-adapted microorganisms therefore represents a sustainable strategy to mitigate petroleum pollution in extreme environments such as Antarctica and any other cold regions with polar or alpine climate, where low temperatures and nutrient scarcity constrain natural attenuation processes.

1. Introduction

Antarctica, one of the coldest environments on Earth, is defined by some extreme abiotic stressors, including sustained subzero temperatures, oligotrophic conditions, sometimes high levels of desiccation, and elevated UV radiation (Goldenberg-Barbosa et al., 2025). Despite these severe constraints, it hosts remarkably diverse and specialized microbial (bacterial, archaeal and fungal) communities, which have adapted their physiology to such harsh conditions (Larsen et al., 2024). Among fungi, cold-adapted yeasts are characterized by biochemical and physiological adaptations, such as the synthesis of cold-active enzymes, the modification of fatty acid profiles of phospholipid membranes and the activation of stress responses that enable survival and even growth and metabolic activity under multiple environmental stressors (Feller and Gerday, 2003; Buzzini et al., 2012, 2018, 2025). Recent structural studies have also begun to elucidate the molecular basis of yeast adaptation to stressful conditions. For instance, Wu et al. (2024) provided structural and functional insights into the telomeric repeat-binding factor Tbf1, highlighting the role of telomere-associated proteins in genome stability under demanding cellular conditions. These

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physiological and metabolic traits make cold-adapted yeasts promising candidates for various industrial and biotechnological applications (Margesin and Schinner, 1994, 1999; Margesin et al., 2008; Buzzini and Margesin, 2014; Sibirny, 2025), including bioremediation of cold contaminated sites (Hua et al., 2004; Tsuji et al., 2013).

In Antarctica, where research and exploration efforts have contributed to localized environmental disturbance, hydrocarbon contamination has emerged as one of the most critical and closely monitored issues (Aislabie et al., 2004; Tin et al., 2009). Hydrocarbons represent a major class of environmental pollutants targeted by bioremediation strategies (Ghosal et al., 2016; Varjani, 2017). Despite their economic importance, hydrocarbons exert significant adverse effects on both environmental and human health (Thomas et al., 2002; Varjani and Upasani, 2017; Varjani et al., 2017). Long-term ecological damage is frequently associated with anthropogenic activities such as oil extraction, transportation, refining and use (Das and Chandran, 2011; Bachmann et al., 2014; Varjani and Upasani, 2017; Ossai et al., 2020).

Hydrocarbon contaminants can originate from different petroleum-derived products with distinct chemical compositions and environmental behavior. Crude oil is a naturally occurring complex mixture containing alkanes, cycloalkanes, aromatic hydrocarbons, resins, and asphaltenes, whereas refined fuels such as gasoline, diesel, and kerosene consist mainly of lower molecular weight hydrocarbons that are generally more volatile and more readily biodegradable (Fowzia and Fakhruddin, 2018). In contrast, lubricating oils are composed predominantly of high molecular weight hydrocarbons and are enriched with additives such as antioxidants, dispersants, detergents, and anti-wear compounds designed to enhance engine performance (Wang et al., 2000). After use in engines, these lubricants become spent engine oil (SEO), a waste product containing degraded hydrocarbons, oxidized compounds, polycyclic aromatic hydrocarbons (PAHs), and trace metals such as zinc, lead, and chromium derived from engine wear (Agarry and Ogunleye, 2012). Due to its chemical complexity and the presence of additives, SEO represents a particularly persistent and environmentally problematic contaminant that can be more recalcitrant to microbial degradation than many other petroleum-derived hydrocarbons (Ibrahim and Salisu, 2024).

Bioremediation is an environmentally sustainable strategy that employs plants and microorganisms, namely bacteria, fungi (including yeasts), and algae to degrade some forms of pollutants (Margesin, 2014; Kalia et al., 2022). According to some authors (Ansari et al., 2023; Tsai et al., 2025), approaches to environmental remediation, particularly for hydrocarbon-contaminated sites, include various methods: (i) natural attenuation, (ii) biosorption, (iii) bioaugmentation and (iv) biostimulation, (v) phytoremediation, (vi) rhizoremediation, and (vii) bioaccumulation. These biologically-based approaches may offer a sustainable and environmentally friendly alternative to conventional physicochemical remediation techniques, such as incineration, thermal desorption and extraction (Varjani, 2017). Moreover, remediation activities in polar and ice-covered environments are often constrained by significant physical and engineering challenges, where even basic operations such as ice breaking can require complex mechanical interventions (e.g., high-speed water jets) to fracture ice layers (Yuan et al., 2022), highlighting the logistical difficulties associated with conventional technological approaches in frozen environments.

In this framework, the use of yeasts may represent a cost-effective and eco-friendly alternative to conventional non-biological methods of hydrocarbon remediation (Das and Chandran, 2011), which are often associated with several limitations, including low efficiency, high operational costs, and the generation of secondary wastes such as sludges (Varjani, 2017; Varjani and Upasani, 2017). The efficacy of yeast-based remediation has been demonstrated in numerous studies (Kumari and Abraham, 2011; Margesin, 2014; Gargouri et al., 2015; Okerentugba et al., 2016; Mohiuddin et al., 2024) through mechanisms such as biosorption, bioaccumulation, biosurfactant production, and enzymatic degradation. These mechanisms enhance hydrocarbon bioavailability and facilitate their transformation into less toxic metabolites.

Yeasts capable of hydrocarbon degradation encompass a functionally and taxonomically diverse group, including members of the genera *Candida, Debaryomyces, Lodderomyces, Metschnikowia, Pichia, Saccharomyces, Trichomonascus* (*Stephanoascus*), and *Yarrowia* within the phylum Ascomycota, and *Leucosporidium, Rhodosporidium, Rhodotorula, Sporidiobolus, Sporobolomyces, and Trichosporon* within the phylum Basidiomycota (Kannan et al., 1990; Csutak et al., 2010; Kumari and Abraham, 2011; Jain, 2012; Zinjarde et al., 2014; Gargouri et al., 2015; Mbachu et al., 2016). Despite the large number of hydrocarbon-degrading yeast genera isolated from various environments (e.g. soil, marine sediments, and petroleum-contaminated sites), only a few have been reported from Antarctic environments. Documented isolates include, the species *Moesziomyces antarcticus* (former *Candida antarctica*) (Hua et al., 2004), *Mrakia blollopis* (Tsuji et al., 2013), *Meyerozyma* (*Pichia*) *caribbica* (Joshi-Navare et al., 2014), *Exophiala macquariensis* (Zhang et al., 2019) and *Phenoliferia glacialis* (Azman et al., 2024).

Although in recent years cold-adapted yeasts have attracted increasing attention due to their potential biotechnological applications at low temperature, a significant number of Antarctic yeasts remain poorly characterized, especially regarding their ability to degrade some classes of hydrocarbons. In this context, microbial degradation has been widely investigated for crude oil and refined petroleum fuels, whereas considerably less attention has been given to lubricant-derived wastes such as spent engine oil (Mekonnen et al., 2024). Current knowledge on SEO biodegradation is mainly based on recent studies involving bacteria (Ismail et al., 2014; Raju et al., 2017) and filamentous fungi (Ganesh Kumar et al., 2021; Lawal et al., 2025) isolated from contaminated environments, whereas, to date the ability of yeasts to utilize or degrade SEO has received little attention and remains largely unexplored. This knowledge gap highlights the need for comprehensive investigation, as these microorganisms may harbor unique metabolic pathways and enzymatic adaptations. Exploring their functional potential as hydrocarbon degraders could contribute to the discovery of novel traits relevant to bioremediation and other biotechnological processes in extreme environments.

From an ecological perspective, microbial communities inhabiting extreme environments can be explained through the framework of r/K selection theory, which describes contrasting life-history strategies shaped by resource availability and environmental stability. r-strategists are typically characterized by rapid growth, high reproductive rates, and the ability to exploit transient or nutrient-rich conditions, whereas K-strategists tend to grow more slowly and are adapted to persist under resource-limited and stable environments (Andrews and Harris, 1986; Fierer et al., 2007). Antarctic terrestrial ecosystems are generally oligotrophic and environmentally harsh, conditions that often favor microorganisms with K-selected traits such as metabolic efficiency, stress tolerance, and the capacity to utilize complex or recalcitrant substrates. However, localized disturbances, including hydrocarbon contamination associated with human activities, particularly during the Antarctic summer season, when most research campaigns are conducted, may introduce nutrient-rich substrates that temporarily favor r-strategist microorganisms capable of rapid growth and opportunistic substrate utilization. Understanding the balance between these ecological strategies may therefore provide valuable insight into microbial responses to hydrocarbon contamination and their potential role in bioremediation processes in polar environments.

In this framework, the present study aimed to: (i) isolate and identify cold-adapted yeasts from Antarctic soils through a hydrocarbon-based enrichment protocol at low temperature; (ii) evaluate their metabolic versatility on different C and N sources at 10 °C; and (iii) investigate their capacity to degrade spent engine oil under cold conditions.

The study highlights their potential for biotechnological applications, with a particular focus on low temperature SEO degradation that could be applied to contaminated soils with average temperatures

around zero. Furthermore, it addresses the urgent need for effective biodegradation agents in polar regions, where persistent hydrocarbon contamination, including SEO contamination, from localized human activities, especially near scientific stations, is exacerbated by extreme environmental conditions that limit natural attenuation processes (Bargagli and Rota, 2024).

2. Materials and methods

2.1. Yeasts isolation

Yeasts were isolated from soil samples collected during the 2020/2021 Italian Antarctic expeditions from two remote sites of Victoria Land (Fig. S1): Kay Island (74°06.99′ S, 165°91.23′ E) and Morris Basin (75°39.483′ S, 159°04.189′ E, 850 m a.s.l.). Soil samples were collected from the 0–10 cm surface layer, corresponding to the biologically active horizon of Antarctic soils where microbial communities are typically concentrated. These two sites are well differentiated from each other, in terms of their physicochemical properties (Table S1). The former is a coastal site, with well-developed biological crusts active during the austral summer, while the latter is an inland site, south of David Glacier, free of snow cover in summer and lacking apparent forms of life.

The isolation protocol was based on methods described by Asemoloye et al. (2020) and Daccò et al. (2020a), with modifications for soil-based applications. Briefly, 5 g of each soil sample from continental Antarctica were suspended in 25 mL of sterile liquid Bushnell-Haas Broth (composition; Magnesium Sulphate: 0.20 g/L, Calcium Chloride: 0.02 g/L, Monopotassium Phosphate: 1.00 g/L, Dipotassium Phosphate: 1.00 g/L, Ammonium Nitrate: 1.00 g/L, Ferric Chloride: 0.05 g/L) and supplemented with 0.25 mL of spent engine oil as the sole C source, to promote the selective growth of cold-adapted yeasts capable of hydrocarbon degradation. Each soil sample was incubated in triplicate in 50 mL flasks at 10 °C under continuous agitation (80 rpm) for 1 month and optical changes in the medium associated with oil transformation were monitored weekly. Yeasts were subsequently isolated by plating 1 mL of the enrichment suspension onto Potato Dextrose Agar (PDA: potato extract 4 g/L, dextrose 20 g/L, agar 15 g/L) and Sabouraud Dextrose Agar (SDA: peptone 20 g/L, dextrose 20 g/L, agar 15 g/L), followed by incubation at 10 °C.

All studied strains are preserved at MicUNIPV-AF – Pavia University Culture Collection Amico Fungo, Pavia, Italy.

2.2. Molecular identification

DNA extraction from the isolated strains was performed using the FastDNA™ SPIN Kit following the manufacturer's protocol. Amplification of the fungal internal transcribed spacer (ITS) regions 1 and 2 was performed using the primers ITS1 (5′ TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), according to (Martin and Rygiewicz, 2005). The PCR products were sequenced by BMR Genomics (Padova, Italy). Yeasts identification was carried out using BLAST searches against the National Center for Biotechnology Information (NCBI) GenBank sequence database (http://www.ncbi.nlm.nih.gov/BLAST/index.html), to obtain the closest type strains sequence matches for taxonomic identification. The most similar sequences were exported; the sequence alignment (Muscle) was generated using Mega7 (Kumar et al., 2016). Phylogenetic tree reconstruction using the maximum-likelihood algorithm was performed, and Tamura 3-parameter model was used, as suggested by the implemented model test. The robustness of the phylogenetic inference was estimated using the bootstrap method with 1000 pseudo-replicates generated.

2.3. Growth tests

Growth assimilation tests were performed according to the protocols described by Kurtzman et al. (2011) using 30 C sources and 5 N sources (listed in Table S2). Yeast isolates were pre-cultured on YPDA medium (1% yeast extract, 1% peptone, 2% dextrose, 2% agar; Oxoid) at 10 °C for 48-72 h. Standardized cell suspensions (10^6 cells/mL), prepared by direct microscopic enumeration, were used to inoculate 96-well microplates containing yeast N base (YNB; Difco) or yeast C base (YCB; Difco) and individual test substrates. The micro-plates were incubated at 10 °C for 14 days. Three replicates for each condition were tested.

Cell growth was assessed by measuring absorbance at 580 nm (A_{580}) using a Tecan Infinite M200 microplate spectrophotometer (Tecan Group Ltd., Switzerland). Growth indices (GI) were calculated from OD_{580} measurements using the equations reported below:

$$GI = \frac{(Absf - Absi)}{Absi \times t}$$

where Abs_f and Abs_i represent the absorbance at 580 nm at the final and initial time points, respectively, and t is the incubation time in days.

$$GIT_3 = \frac{Abs_3 - Abs_0}{Abs_0 \times 3} \quad GIT_7 = \frac{Abs_7 - Abs_3}{Abs_3 \times 4} \quad GIT_{14} = \frac{Abs_{14} - Abs_7}{Abs_7 \times 7}$$

where Abs_0, Abs_3, Abs_7 Abs_{14} represent absorbance at 580 nm measured at the starting point and after 3, 7 and 14 days of incubation. GIT_3, GIT_7, and GIT_{14} represent interval-based growth indices describing the relative increase in optical density per day within each incubation interval. This approach enables the assessment of temporal growth dynamics under low-temperature conditions and facilitates the comparison of strain-specific responses across different incubation phases. This index provides a sensitive and quantitative measure of metabolic activity, which is mainly relevant for cold adapted yeasts, where growth is usually slow and strongly influenced by environmental constraints. Growth was considered effective when the growth index (GI) exceeded the threshold of 0.2 ($GI > 0.2$), a value selected to reflect active metabolic activity, while reducing the impact of inherent variability in optical density measurements arising from incubation conditions or instrumental noise (Biesta-Peters et al., 2010).

2.4. Hydrocarbon degradation analysis

The chemical composition of the spent engine oil used in this study was characterized by gas chromatography–mass spectrometry (GC-MS) and used as a control to assess the ability of selected yeast strains to degrade spent engine oil in Bushnell-Haas (BH) broth, following the protocol of Daccò et al. (2020b). Chemical analysis was performed by the private company KCS biotech (https://kcsbiotech.it/). A volume of 25 mL of BH medium was supplemented with 1% (v/v) spent engine oil and inoculated with each yeast strain (10^5 cells/mL), while flasks without yeast inoculation served as controls. The flasks were incubated at 10 °C under continuous agitation at 80 rpm for 30 days. Flasks were closed with cotton plugs wrapped in aluminum foil to allow aerobic gas exchange while minimizing contamination. Parallel abiotic control flasks containing BH medium and spent engine oil, but no yeast inoculum, were incubated under the same conditions and used to assess possible non-biological losses, including potential volatilization of lighter hydrocarbon fractions. The experiment was conducted in triplicate.

The efficiency of hydrocarbon degradation was determined using GC-MS after 30 days. Briefly, samples were uniformly recovered from the oil phase adhering to the glass after homogenization by gently shaking the flasks on an orbital shaker (60 rpm, room temperature, 5 min) followed by phase separation at 4 °C for 30 min. This step was used to homogenize the residual oil-biofilm mixture before sampling. A 15 mg extract of the residual oil phase was then collected and mixed with 40 μL of TBDMS derivatizing agent. The samples were silylated overnight at 20 °C in darkened, teflon-sealed tubes. Silylated solutions were prepared at a concentration of 10 mg/mL in ethyl ether, with 10 mM thymol added as an internal standard. The solutions were further diluted

in pure ethyl ether (Merck, GC-grade), dried over Na_2SO_4, and filtered through 0.2 μm nylon microfilters immediately before injection. Two microliters of each sample were injected, and the GC method (Clarus 600, PerkinElmer) is briefly described as follows: initial temperature of 40 °C for 1 min, followed by two ramped steps, first to 120 °C at a rate of 5 °C/min, and then to 280 °C at a rate of 8 °C/min, and a final holding at 280 °C for 5 min. Elution was performed on a TR5-MS column (15 m × 250 μm, 1.5 mL/min flow rate), using helium as the carrier gas. The injector was set to splitless mode at 280 °C. The MS method (Clarus SQ 8, PerkinElmer) is described as follows: scanning range 35–800 m/z (EI+), full scan, 0.4 scans per second, with a 5-min delay. The ion source was set to 220 °C, and the transfer line to 280 °C.

Peaks obtained from the GC-MS analysis were measured using the Total Ion Current (TIC) area, and chemical classes were assigned based on marker peaks, following the method of Hostettler et al. (2013). Peak areas were normalized using an internal standard and used to calculate the compositional percentage of each chemical class within the hydrocarbon mixture. This approach was adopted to evaluate relative changes in mixture composition among treatments, while comparisons with the abiotic control were used to interpret variations in TIC signal intensity associated with degradation processes. A cutoff value for each chemical class in each sample was derived from library analysis and used to explore the multitude of recorded peaks, selecting those most suitable for comparison with the NIST18 library.

Only compounds identifiable in the library with a Reverse Score (Rev. score) > 85% were selected. The sum of the TICs for these characterized compounds was considered the total sample area (TIC Sample). The TIC of each peak was compared to the corresponding compound (based on retention time and identification) in the control sample. The sum of the TICs for compounds within the same class was compared to the TIC of the same class in the control sample. Each sample was considered as a whole and expressed as a compositional percentage based on the TIC of its individual chemical class components.

2.5. Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 10.4.1 for Windows (GraphPad Software, Boston, Massachusetts, USA). To determine whether the results were significantly different, data were analyzed using one-way ANOVA at 95% of the significance, followed by Tukey's honest significance difference (HSD) post hoc test. Results were considered statistically significant at $p < 0.05$. These statistical tests were applied to the relative abundance values of each hydrocarbon class obtained from GC-MS analysis, including minor fractions such as BTEX and alkyl biphenyls, using three independent replicates for each treatment. Before ANOVA, normality was assessed with the Shapiro–Wilk test, and homogeneity of variances was evaluated using Bartlett's test. The Venn diagram was generated using the online tool InteractiVenn by Heberle et al. (2015).

3. Results

3.1. Strain identifications

Three distinct yeast strains were isolated from Antarctic soil samples enriched with spent engine oil as the sole C source: MR34, 4KIA, and 2KIC. Molecular identification indicated that the isolates belonged to the species *Rhodosporidiobolus odoratus*, *Rhodotorula mucilaginosa*, and *Filobasidium magnum*, respectively (Table S3). Phylogenetic reconstruction based on ITS1-5.8S-ITS2 sequences placed the strains in three distinct clusters corresponding to the identified species, further confirming their taxonomic position (Fig. 1).

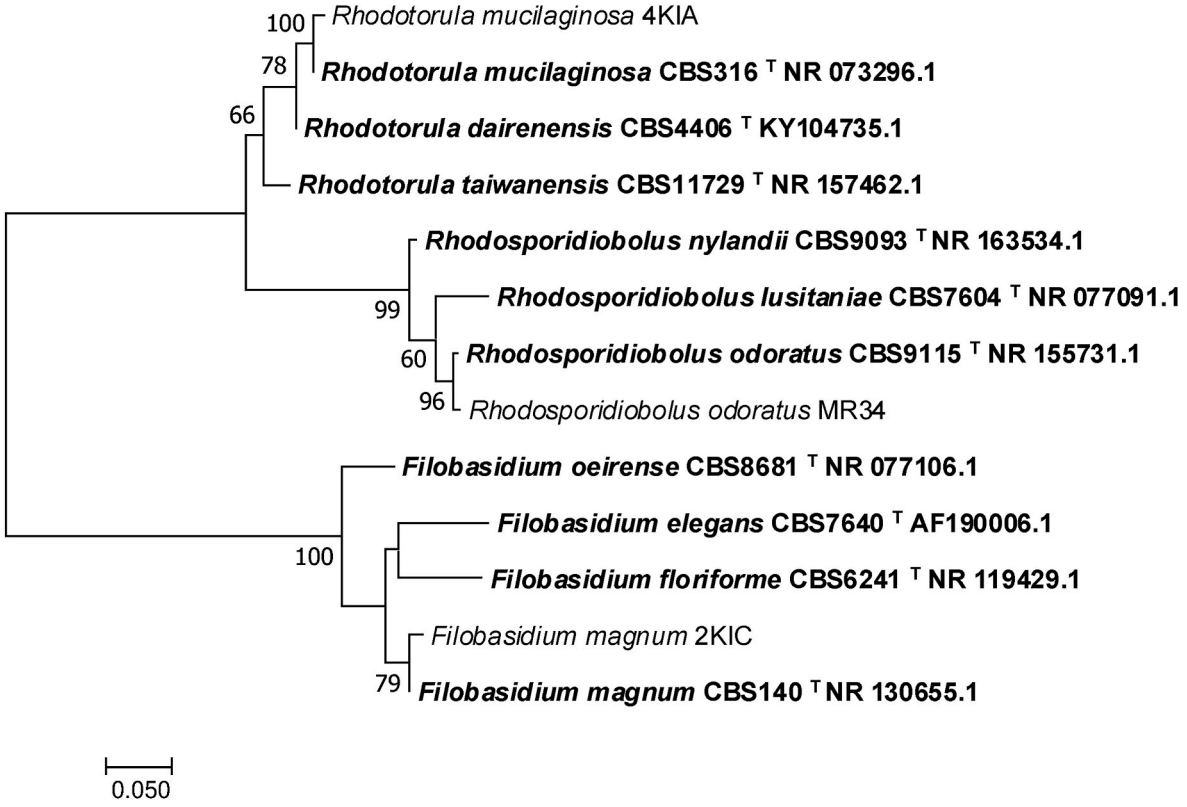

Fig. 1. Maximum likelihood analysis of sequences of the internal transcribed spacer (ITS) regions. The numbers given on branches are frequencies (>50 %) with which a given branch appeared in 1000 bootstrap replications. The scale indicates the number of expected substitutions accumulated per site. Type strains are shown in bold, and GenBank accession numbers follow the strain names.

3.2. Substrates utilization profiles

The tests conducted to evaluate the growth index (GI) of the three strains using a broad set of different C and N sources yielded different responses among them.

R. odoratus exhibited a narrow assimilation profile: a GI > 0.2 was found on eight C sources. Among N sources, only nitrate supported a GI > 0.2 (Fig. 2A). *R. mucilaginosa* demonstrated a broader assimilation profile: a GI > 0.2 was found on twelve C sources. Among N sources, ethylamine supported a GI > 0.2. Notably, most assimilations occurred after three days of incubation, indicating a rapid metabolic response across a diverse range of substrates (Fig. 2A). Finally, *F. magnum* displayed the broadest assimilation profile: a GI > 0.2 was found on fifteen C sources: among them, the assimilation of the hydrocarbon hexadecane was putatively indicative of potential hydrocarbon-degrading capability. *F. magnum* was also the only yeast of this study capable of utilizing L-lysine as a N source. Notably, the major assimilation activity was observed after seven days of incubation, indicating a slower but progressive metabolic engagement with a wide range of substrates (Fig. 2A).

Comparative analysis of substrate assimilation patterns revealed a set of substrates supporting a GI > 0.2 across all three strains (Fig. 2B):

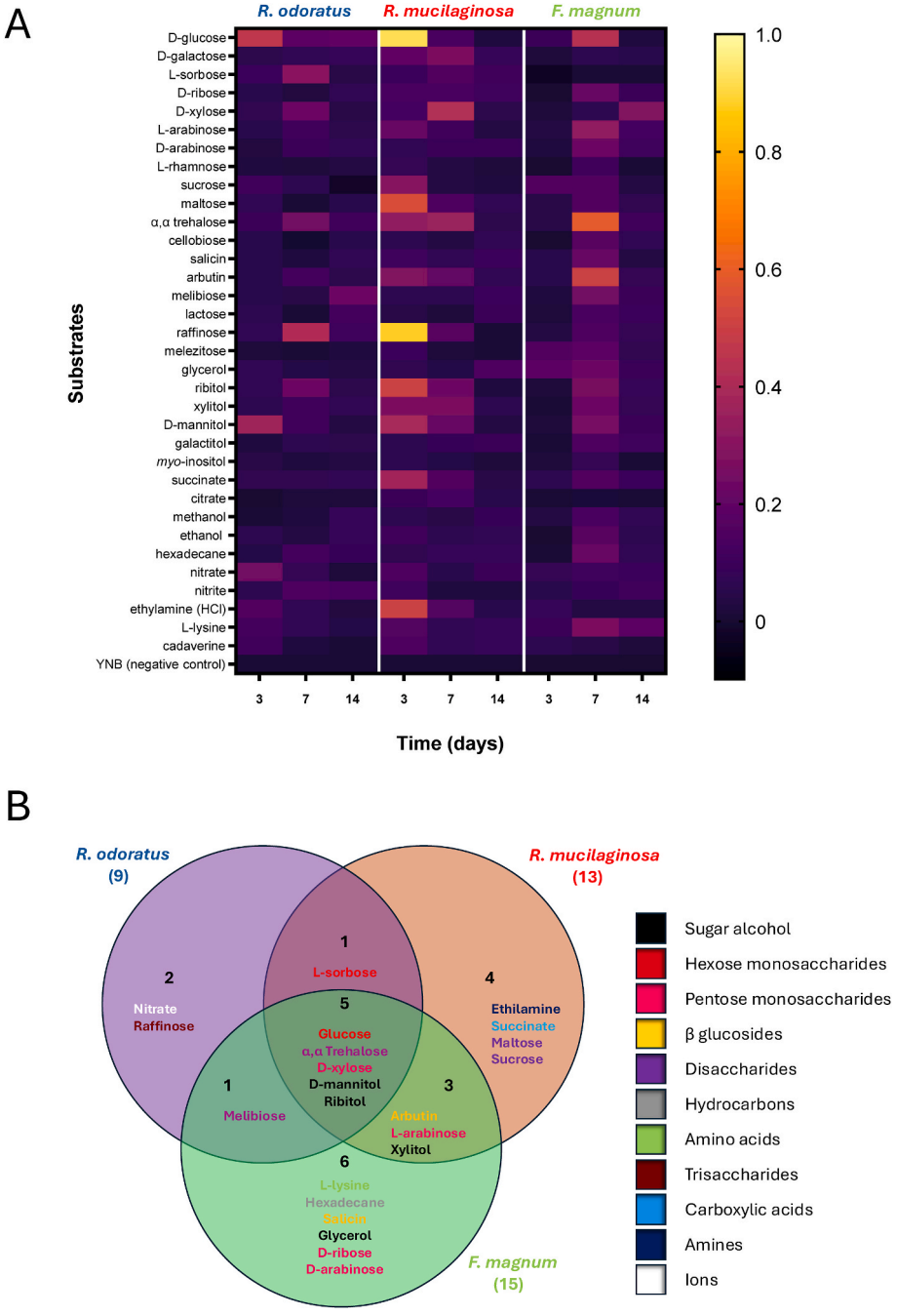

Fig. 2. (a) Heatmap illustrating the differential substrate utilization profiles of the yeast species *R. odoratus* MR34 (blue), *R. mucilaginosa* 4KIA (red), and *F. magnum* 2KIC (green) over an incubation period of 3, 7, and 14 days. The y-axis lists the 41 tested substrates, while the x-axis denotes the incubation times. Color intensity represents relative metabolic activity, with dark purple indicating no substrate utilization and bright yellow denoting high substrate utilization. (b) Venn diagram summarizing the number and identity of substrates utilized by each species. Substrates are color-coded by chemical class as indicated in the legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

glucose, D-xylose, D-mannitol, ribitol, and α,α-trehalose.

3.3. Hydrocarbon degradation

The chemical composition of the spent engine oil used in this study revealed a complex mixture of hydrocarbons and related compounds (Fig. 3). The predominant constituents were C_{20}–C_{50} alkanes, representing 78% of the total composition, indicating a substantial fraction of high-molecular-weight aliphatic hydrocarbons. Methyl esters were the second most abundant group (about 11%), probably derived from lubricant additives or from partial degradation processes. Polycyclic aromatic hydrocarbons (PAHs) accounted for about 5% of the mixture, suggesting the presence of persistent and potentially toxic aromatic compounds. In addition, the set of benzene, toluene, ethylbenzene, and xylene compounds (BTEX), along with alkyl biphenyls, contributed for about 3%. Minor fractions included C_1–C_{20} alkanes (2%) and a combination of alkylbenzenes and indenes (1%; Fig. 3).

The flasks containing spent engine oil and inoculated with the three different yeast cultures showed distinct visual characteristics (Fig. S2). GC-MS profiling of spent engine oil after 30 days of incubation of three tested strains revealed significant ($p < 0.05$) differences in the residual composition of hydrocarbon compound classes (Fig. 4). The total residual hydrocarbon abundance (Fig. 4A) was significantly ($p < 0.05$) lower after growth of *R. odoratus*, indicating a significantly ($p < 0.05$) higher degradation efficiency. On the contrary, both *R. mucilaginosa* and *F. magnum* showed no significant ($p \geq 0.05$) reduction in hydrocarbon load compared to the control, after incubation.

Analysis of specific hydrocarbon classes demonstrated distinct degradation patterns among the microbial strains. Considering the short-chain alkanes (Fig. 4B), all three yeast strains exhibited a significant ($p < 0.05$) higher relative abundance of C_1–C_{20} compounds compared to the control (244%, 230% and 173.5% respectively), indicating a relative compositional shift consistent with the breakdown of high-molecular-weight alkanes into lower-molecular-weight fractions. In contrast, the content of long-chain alkanes (Fig. 4C), which are the main components of the spent engine oil, was significantly ($p < 0.05$) lower in samples incubated with *R. odoratus* compared to the control, indicating a significantly ($p < 0.05$) higher degradation efficiency, differently from both *R. mucilaginosa* and *F. magnum*, which exhibited no significant ($p \geq 0.05$) reduction of C_{20}–C_{50} alkane content compared with the control. *R. odoratus* also demonstrated a superior degrading ability towards methyl esters content (Fig. 4D), which significantly ($p < 0.05$) decreased after 30 days of incubation.

Concerning the aromatic fractions, both *R. odoratus* and *F. magnum* significantly ($p < 0.05$) reduced PAHs (Fig. 4E), as well as alkyl benzenes and indenes (Fig. 4F), compared to the abiotic control.

In addition, *F. magnum* was particularly efficient in degrading BTEX and alkyl biphenyls, which resulted in the lowest residual fraction (55%), significantly lower than those observed in the other strains and the control (Fig. 4G). In contrast, a significant relative increase ($p < 0.05$) in BTEX and alkyl biphenyls was observed after incubation with *R. odoratus* and *R. mucilaginosa*, compared to the control (Fig. 4G).

4. Discussion

Hydrocarbon contamination has increasingly been recognized as a critical environmental issue in cold climate regions, due to the higher vulnerability of these environments compared to temperate and tropical regions (Filler et al., 2008). This greater vulnerability arises mainly from harsh conditions, including low temperatures, nutrient limitation, and recurrent freeze–thaw cycles, which contribute to slower rates of natural attenuation of contamination and allow oil pollutants to persist in the environment for more than 20 years (Yap et al., 2021). Furthermore, remoteness, lack of appropriate infrastructure, extreme climatic conditions in the cold regions worldwide (including polar ones), and specific physical and chemical properties of cold soils may reduce the effectiveness and limit the applicability of conventional remediation technologies (Naseri et al., 2014). Therefore, the development of clean-up strategies, such as bioremediation using culturable cold-adapted hydrocarbon-degrading yeast strains, could represent a promising and cost-effective approach for the remediation of hydrocarbon-polluted cold environments (Margesin, 2014).

The results of this study provide novel insights into the physiological responses to hydrocarbon exposure exhibited by three cold-adapted yeasts isolated from Antarctic soil and cultured in the presence of spent engine oil, highlighting differing degradation strategies at low temperatures.

F. magnum showed the highest growth with hexadecane as the sole C source (Fig. S3), while *R. mucilaginosa* and *R. odoratus* had significantly lower optical density (OD) and growth index (GI), indicating limited ability to metabolize hexadecane (Fig. S3A and S3B). *R. odoratus,* despite not growing on hexadecane, degraded different classes of hydrocarbons (especially aliphatic compounds) efficiently. These findings suggest that hexadecane may not assess yeast hydrocarbon degrading ability, as it mainly indicates mid-chain alkanes breakdown (e.g., C_{16}) rather than broader aliphatic hydrocarbon. Previous studies proposed hexadecane as a marker for hydrocarbon assimilation (Cirigliano and Carman, 1984; Ulfig et al., 2007; Trama et al., 2014; Gargouri et al., 2015), but the results reported herein suggest it may underestimate degradative potential. In particular, *R. odoratus* showed no growth on hexadecane, but exhibited the highest degradation efficiency toward long-chain alkanes (C_{20}–C_{50}) in spent engine oil, highlighting that hexadecane is not universally predictive of aliphatic hydrocarbon degradation performance.

This apparent discrepancy may reflect differences between growth on pure hexadecane as the sole carbon source and transformation of long-chain alkanes within a chemically complex oil mixture. In yeasts, alkane assimilation depends on inducible oxidative systems, including cytochrome P450 alkane hydroxylases, whose expression is regulated by alkane-responsive transcriptional networks and may vary according to substrate composition (Endoh-Yamagami et al., 2007; Hirakawa et al., 2009). Moreover, alkane hydroxylases can differ in chain-length preference, and degradation of long-chain alkanes may involve enzymatic

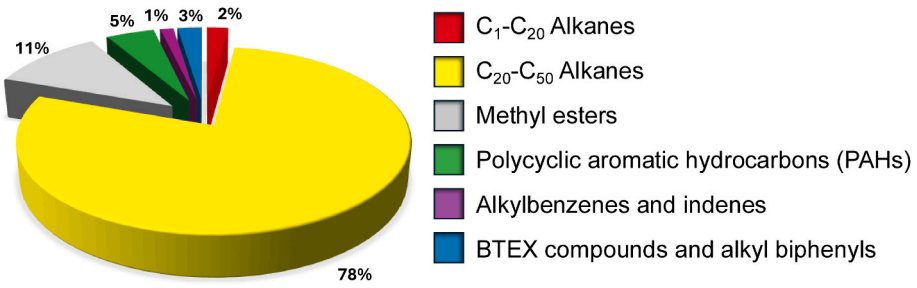

Fig. 3. GC-MS-based compositional profile of spent engine oil, showing the relative abundance of major hydrocarbon fractions. The analysis reveals C_{20}–C_{50} alkanes as the predominant class (78%), followed by methyl esters (11%), polycyclic aromatic hydrocarbons (5%), BTEX and alkyl biphenyls (3%), C_1–C_{20} alkanes (2%), and alkyl benzenes and indenes (1%).

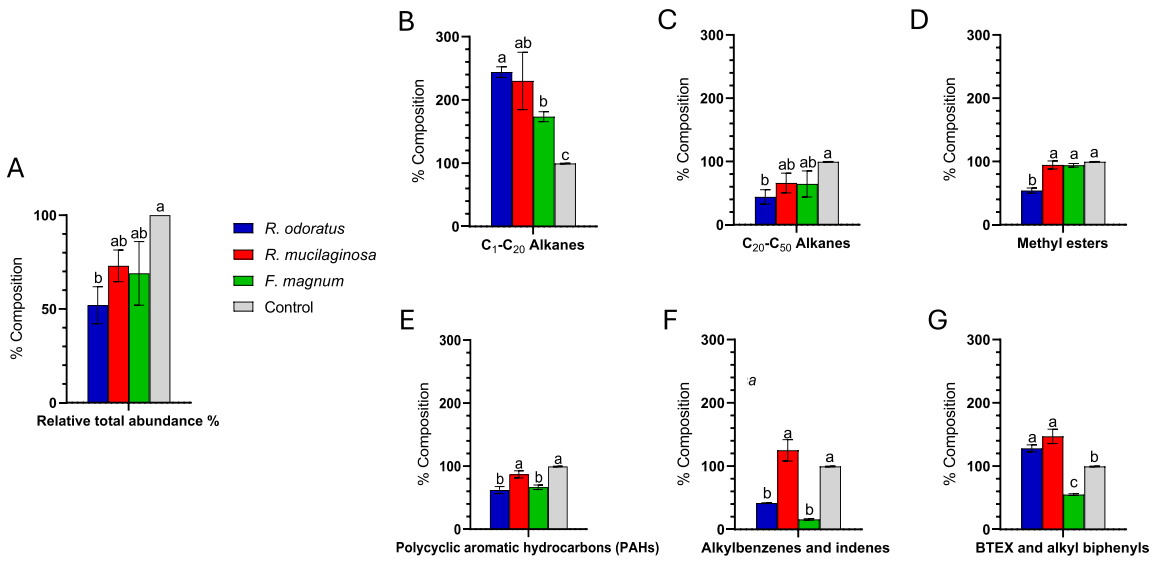

Fig. 4. Composition of hydrocarbon compound classes detected by GC-MS after 30-day incubation of spent engine oil with *R. odoratus* MR34, *R. mucilaginosa* 4KIA, *F. magnum* 2KIC, and the abiotic control. Panels show: (A) Relative total residual hydrocarbon abundance, (B) short-chain alkanes (C_1–C_{20}), (C) long-chain alkanes (C_{20}–C_{50}), (D) methyl esters, (E) polycyclic aromatic hydrocarbons (PAHs), (F) alkylbenzenes and indenes, and (G) BTEX and alkyl biphenyls. Values are expressed as mean ± standard deviations (SD). Data were analyzed by one-way ANOVA with post hoc pairwise comparisons. Different lowercase letters indicate statistically significant differences among treatments within each compound class ($p \leq 0.05$).

systems that are not efficiently triggered by hexadecane alone (Ji et al., 2013; Wang et al., 2024). Therefore, the complex composition of spent engine oil may have promoted the induction of oxidative pathways, cometabolic transformation, and interfacial growth, allowing *R. odoratus* to efficiently degrade C_{20}–C_{50} hydrocarbons despite the absence of detectable growth on hexadecane in the screening assay.

Mechanisms of stress adaptation and ecological specialization related to specific ecological niche, like substrate-specific traits associated with the isolation source or with the growth on substrates such as spent engine oil, can significantly affect biodegradation efficiency and should be carefully evaluated when selecting strains for bioremediation applications.

GC-MS results showed that *R. odoratus* efficiently degrades hydrocarbons in spent engine oil, including long-chain aliphatic hydrocarbons (C_{20}–C_{50}) and complex compounds, such as methyl esters, which together accounted for approximately 89% of the total chemical composition of spent engine oil. *R. odoratus* also degraded aromatic compounds such as PAHs, alkylbenzenes, and indenes, but showed incomplete degradation of certain intermediates, especially BTEX compounds.

Although *R. odoratus* exhibited a superior ability to degrade hydrocarbons, the tested strains use different degradation pathways of hydrocarbons in spent engine oil. The observed higher relative abundance of C_1–C_{20} alkanes, detected in all yeasts under study after incubation on spent engine oil, especially *R. odoratus* and *R. mucilaginosa*, is consistent with the partial degradation of long-chain alkanes (C_{20}–C_{50}). This suggests a terminal or subterminal oxidation followed by β-oxidation, resulting in the relative enrichment of shorter-chain alkanes, potentially as intermediate or partial degradation products (Adedeji et al., 2022). The reduction in C_{20}–C_{50} alkanes supports this interpretation, with *R. odoratus* showing the highest efficiency, consistent with its elevated accumulation of short-chain products.

The significant reduction of methyl esters in spent engine oil after incubation with *R. odoratus* may indicate esterase activity, enabling cleavage into fatty acids and methanol (Nuylert and Hongpattarakere, 2013). The high level of methyl esters hydrolysis exhibited by *R. odoratus* (substantially 54% reduction) is higher than that reported for *Papiliotrema* (former *Cryptococcus*) *laurentii* and *Clavispora* (*Candida*) *lusitaniae*, both isolated from mangrove forest areas (Syah et al., 2018). In particular, *C. lusitaniae* showed an approximately 30–40% reduction of methyl esters after incubation in diesel oil (Syah et al., 2018).

Both *R. odoratus* and *F. magnum* exhibited the ability to degrade polycyclic aromatic hydrocarbons (PAHs). This capability is often associated with the release of mono- or dioxygenase enzymes capable of initiating aromatic ring cleavage (Padilla-Garfias et al., 2024). Interestingly, *R. mucilaginosa* showed a marked relative increase in alkylbenzenes and indenes (125% increase). This observation may suggest a partial transformation of higher molecular weight aromatic compounds, such as alkylated PAHs or biphenyls, into simpler monoaromatic hydrocarbons. The accumulation of these monoaromatic intermediates may also have toxic effects on microbial cells. Aromatic hydrocarbons such as BTEX are known to disrupt membrane structure, interfere with cellular respiration, and inhibit enzymatic activity in microorganisms when degradation pathways are incomplete (Das and Chandran, 2011; Varjani and Upasani, 2017). The relative enrichment of these intermediates observed for *R. mucilaginosa* may therefore partially explain the limited biomass production and the absence of evident emulsification or interfacial colonization compared with the other strains. In such conditions, the strain may initiate the transformation of complex hydrocarbons but fail to efficiently metabolize the resulting aromatic intermediates, leading to metabolic inhibition and reduced biodegradation efficiency. Similar patterns have been reported in fungi with limited ability to break aromatic rings, where partial breakdown of complex compounds results in the accumulation of simpler but still persistent aromatic intermediates (Prenafeta-Boldú et al., 2019). Conversely, the incubation of spent engine oil with *R. odoratus* and *F. magnum* not only reduced PAH levels, but also significantly depleted alkylbenzenes and indenes (83% and 58%, respectively), suggesting the presence of more complete degradation pathways capable of processing both primary hydrocarbons and their aromatic intermediates.

A similar trend was observed for BTEX compounds: *F. magnum* significantly reduced BTEX levels (45%), while *R. odoratus* and *R. mucilaginosa* exhibited notable relative increases in BTEX abundance of 128% and 147%, respectively. These contrasting trends suggest differences in enzymatic degradation potential among the yeasts utilized in this study. The reduction of BTEX levels in spent engine oil after incubation with *F. magnum* may suggest the activity of oxidative enzymes, such as monooxygenases, dioxygenases, and possibly laccases able to

catalyze the breakdown of BTEX into catecholic or quinonoid intermediates, thereby enabling further metabolism through central C pathways (Reineke and Schlömann, 2023; Abdelhamid et al., 2024). In contrast, the higher relative abundance of BTEX observed in spent engine oil after incubation with *R. odoratus* and *R. mucilaginosa* may indicate incomplete degradation, potentially due to limited expression or absence of such oxidative enzymes. Thus, both above yeasts could perform only partial transformation of specific complex aromatic compounds, leading to the relative enrichment of BTEX-like compounds with the residual hydrocarbon mixture (Abdelhamid et al., 2024). This pattern may also reflect broader metabolic constraints associated with the degradation of aromatic hydrocarbons by cold-adapted yeasts. The initial activation of BTEX compounds requires oxygenase-mediated reactions that introduce oxygen into the aromatic ring, a process that is metabolically demanding and often less efficient at low temperatures (Das and Chandran, 2011; Sathesh-Prabu et al., 2023). Moreover, compared with bacteria, yeasts generally possess fewer specialized pathways for the degradation of monoaromatic hydrocarbons, which may result in slower transformation rates or incomplete mineralization of these compounds in cold environments (Padilla-Garfias et al., 2024).

The visual appearance of spent engine oil (Fig. S2) reflected the distinct metabolic capacities of the tested strains and was consistent with the differential hydrocarbon degradation assessed by GC-MS. After three weeks of incubation, *R. odoratus* showed clear growth in the hydrocarbon phase (Fig. S2D), with an adherent biofilm ring at the flask border suggesting colonization at the oil–aqueous interface, a key zone for hydrocarbon access and degradation. In addition, the medium turbidity and uneven emulsion could imply partial emulsification, probably mediated by extracellular release of biosurfactants or exopolysaccharides (EPS), consistent with the EPS-producing phenotype recently described for *R. odoratus* by Zhao et al. (2023). The orange-yellow pigmentation observed after incubation confirmed the growth of *R. odoratus* together with a concomitant pigment production under hydrocarbon stress (Fig. S2D). In contrast, *R. mucilaginosa* showed low turbidity, minimal surface and wall deposits, and limited color change (Fig. S2H), consistent with its inability to degrade major classes of hydrocarbon, as revealed by GC-MS. Although *R. mucilaginosa* is known to produce emulsifying EPS (Mohammed et al., 2021), the sharp oil–water interface and absence of visible interfacial structures suggest only transient dispersion rather than sustained metabolic degradation. Conversely, *F. magnum* exhibited low turbidity, but a structured net at the oil–water interface and moderate wall-associated deposits (Fig. S2L), consistent with degradation of specific hydrocarbon fractions. The reticulated biofilm-like matrix at the interface indicates surface-associated colonization facilitating access to hydrophobic compounds. This behavior may be supported by EPSs production (Vadkertiová et al., 2017) and by the lipolytic and proteolytic activities reported for this species (Văcar et al., 2022), which could enhance adhesion and nutrient acquisition. Overall, these observations suggest a surface-bound biodegradation strategy, with metabolic activity concentrated at the oil-water interface (Fig. S2K–L).

Based on the above different patterns, it is possible to postulate an ecological interpretation of the potential role of the environmental yeasts under study in a hypothetical cold soil contaminated by hydrocarbons on the basis of the ecological interpretation on r/K-selection theory formulated by Fierer et al. (2007) for bacteria. Following this theory, the physiological traits observed for *F. magnum*, including broad substrates assimilation and slower but sustained metabolic activity during incubation, are consistent with K-strategist traits, such as metabolic efficiency, ecological specialization, and adaptation to resource-limited environments. Its biodegradation strategy likely involves the formation of EPS, which, as underlined above, may enhance emulsification and promote sustained hydrocarbon degradation (Vadkertiová et al., 2017). In contrast, *R. mucilaginosa* shows traits typical of an r-strategist organism (Fierer et al., 2007), favoring rapid colonization and opportunistic substrate transformation, without achieving complete degradation, a strategy consistent with its isolation from Kay Island, where extensive summer moss and lichen mats indicate a dynamic and resource-fluctuating environment. Instead, *R. odoratus* appears to display intermediate ecological traits within the r/K framework, combining selective substrate utilization with an efficiency to degrade complex hydrocarbon fractions. This interpretation is supported by its relatively narrow assimilation profile on simple carbon substrates, together with its ability to transform long-chain hydrocarbons suggesting metabolic specialization toward chemically complex carbon sources rather than rapid exploitation of easily assimilable substrates. Such physiological patterns may reflect adaptation to environments where carbon availability is limited but structurally complex, a condition typical of oligotrophic Antarctic soil.

Overall, these results highlight the potential of cold-adapted yeasts, which may adopt different ecological strategies (r/K selection), as biotechnological agents for the bioremediation of hydrocarbon-polluted environments, where low temperatures can limit natural attenuation and constrain the performance of conventional clean-up strategies. Their physiological characteristics enabling growth under cold conditions and their distinct metabolic traits confer effective hydrocarbon-degrading capabilities and provide valuable insights for developing efficient and sustainable bioremediation approaches applicable to polar regions and other cold environments worldwide.

5. Conclusions

This study highlights the functional diversity of cold-adapted yeasts isolated from Antarctic soils and their ability to transform hydrocarbons under low-temperature conditions. Among the tested strains, *F. magnum* exhibited a broad degradation profile, including several aliphatic and aromatic hydrocarbon fractions present in spent engine oil, whereas *R. odoratus* showed the highest overall degradation efficiency, significantly reducing total hydrocarbon abundance. *R. odoratus* displayed efficient degradation of long-chain alkanes and methyl esters, whereas *R. mucilaginosa* showed limited degradation capacity and accumulated aromatic intermediates. These findings demonstrate that Antarctic yeasts display distinct physiological strategies in transforming components of spent engine oil and represent promising microbial resources for the biodegradation of petroleum-derived contaminants in cold environments.

CRediT authorship contribution statement

Bryan Guerra: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Gianmarco Mugnai:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation. **Ciro Sannino:** Writing – review & editing. **Benedetta Turchetti:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Fabiana Canini:** Writing – review & editing. **Pietro Buzzini:** Writing – review & editing, Supervision, Conceptualization. **Laura Zucconi:** Writing – review & editing, Conceptualization. **Solveig Tosi:** Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2026.106338.

Data availability

Data will be made available on request.

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