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ORIGINAL ARTICLE

Biochemical features and early adhesion of marine *Candida parapsilosis* strains on high-density polyethylene

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Abstract

Aims: Plastic debris are constantly released into oceans where, due to weathering processes, they suffer fragmentation into micro- and nanoplastics. Diverse microbes often colonize these persisting fragments, contributing to their degradation. However, there are scarce reports regarding the biofilm formation of eukaryotic decomposing microorganisms on plastics. Here, we evaluated five yeast isolates from deep-sea sediment for catabolic properties and early adhesion ability on high-density polyethylene (HDPE).

Methods and Results: We assessed yeast catabolic features and adhesion ability on HDPE fragments subjected to abiotic weathering. Adhered cells were evaluated through Crystal Violet Assay, Scanning Electron Microscopy, Atomic Force Microscopy and Infrared Spectroscopy. Isolates were identified as *Candida parapsilosis* and exhibited wide catabolic capacity. Two isolates showed high adhesion ability on HDPE, consistently higher than the reference *C. parapsilosis* strain, despite an increase in fragment roughness due to weathering. Isolate Y5 displayed the most efficient colonization, with production of polysaccharides and lipids after 48 h of incubation.

Conclusion: This work provides insights on catabolic metabolism and initial yeast-HDPE interactions of marine *C. parapsilosis* strains.

Significance and Impact of the Study: Our findings represent an essential contribution to the characterization of early interactions between deep-sea undescribed yeast strains and plastic pollutants found in oceans.

KEYWORDS

biofilms, degradation, diversity, metabolism, yeasts

Maiara Monteiro Oliveira and Audrey Menegaz Proenca contributed equally to this work.

INTRODUCTION

The critical increase of production, consumption and improper disposal of plastic polymers have culminated in a dramatic impact on aquatic and terrestrial life (Lu et al., 2019). Plastic waste represents about 90% of marine pollutants, with floating debris estimated to range from 15 to 51 trillion particles in the ocean (Lacerda et al., 2019; Van Sebille et al., 2015). Polyethylene (PE) is among the most recalcitrant plastic pollutants and constitutes a challenge for biodegradation. High-density polyethylene (HDPE) has a particular impact due to its broad industrial application. HDPE is characterized by over 90% crystallinity and low permeability, with resistance to stress, compression and tensile strength (Coutinho et al., 2003). These properties are retained even when the polymer is synthesized from plant biomass, requiring over 100 years for soil mineralization (Coutinho et al., 2003; Mohsenzadeh et al., 2017; Posen et al., 2015).

Plastic waste—which spreads in the environment as visible fragments (≥5 mm), microplastics (<5 mm) and nanoplastics (<0.1 μm) (Posen et al., 2015)—creates stable surfaces that are colonized by diverse microbial life, often culminating in the formation of biofilms (Keswani et al., 2016; Mohsenzadeh et al., 2017). Biofilms consist of sessile microbial communities that adhere to biotic or abiotic surfaces and produce an extracellular matrix in which they remain embedded. The formation of a biofilm usually starts with the attachment of bacterial cells, which modify physicochemical properties of the substrate and facilitate the adhesion of successive colonizers, such as yeasts, heterotrophic flagellates, diatoms, and ciliates (de Carvalho, 2018). In return, the composition and surface properties of plastic fragments can alter microbial assemblages and their function (Kettner et al., 2017). The natural weathering of polymers in the environment, for instance, increases surface roughness and impacts biofilm formation. Our previous work has shown that the adhesion of marine bacteria to microplastics is impacted by surface weathering, to which bacteria respond by increasing the production of extracellular matrix (Oliveira et al., 2021). In this context, a similar characterization of marine yeast biofilms has not been addressed.

Although aquatic yeasts have been reported as biofilm formers, as previously described for *Candida* spp., *Saccharomyces cerevisiae*, *Pichia kudriavzevii* and *Cryptococcus* sp. (Kutty & Philip, 2008; Wang et al., 2008; Zaky et al., 2016), their adhesion to plastic waste has not been fully characterized. Biofilm formers are able to promote the protection of individual cells against environmental stress, including competition and predation, UV exposure, desiccation, temperature and pH changes, and nutrient shortage (de Carvalho, 2018). Additionally, yeasts exhibit distinctive metabolic attributes, as generally oxidative or fermentative and asporogenous organisms, which renders them desirable for biotechnological and industrial applications (Kutty & Philip, 2008; Wang et al., 2008; Zaky et al., 2016). Marine yeasts are also known to be versatile agents of biodegradation. This happens due to their wide participation in important ecological processes, such as nutrient-recycling and biodegradation of oil/recalcitrant compounds (Kutty & Philip, 2008). *Candida* spp. has been reported as highly adaptable to different environmental conditions (Gadanho & Sampaio, 2005; Kutty & Philip, 2008; Medeiros et al., 2012; Wang et al., 2008; Zaky et al., 2016) including extreme temperatures, hypersaline habitats, and tolerating high osmotic stress (Butinar et al., 2005, 2011; Zaky et al., 2016). Thus, the identification of efficient biofilm-forming yeasts has both environmental and technological implications.

Given the permanent and intense deposition of plastic waste in the oceans, especially in high depths, marine environments became a target for bioprospection of microorganisms with potential for polymer adhesion and degradation (Kane et al., 2020; Lobelle & Cunliffe, 2011; Pathak & Navneet, 2017; Zettler et al., 2013). Here, we evaluate yeast isolates from deep-sea sediment for their biochemical properties, adhesion abilities, and interaction with HDPE fragments subjected to a gradient of weathering treatments.

MATERIALS AND METHODS

Origin of isolates and culturing methods

Marine yeast strains were previously isolated from deep-marine sediment samples collected in 2013 during an oceanographic expedition at the Pelotas Basin (Rio Grande do Sul, Brazil), which has been described as methane seep site (Miller et al., 2015) with the occurrence of a methane-based chemosynthetic metazoan community (Giongo et al., 2016). The sediment samples used for microbial cultures were collected aseptically from a shallow depth (0–3 m below the sea floor), in an area with water column of approximately ~1500 m. Collected samples were stored with 30% glycerol (v/v) at −80°C. These sediment samples contained micro- and nanoplastic fragments visualized by preliminary light microscopy analysis (100–1000× magnification; Light Microscope CX31; Olympus) (unpublished data).

For yeast isolation, sediment samples were inoculated in Yeast Extract-Peptone-Dextrose (YPD) medium (1% yeast extract, 2% peptone and 2% dextrose; Kasvi®), supplemented with 0.05% chloramphenicol (Kasvi®) (v/v) and incubated at 30°C for at least 48 h. These cultures provided

a total of ten yeast isolates that were stored at −80°C with 30% glycerol (v/v).

For our experiments, all isolates (Y1–Y10) were recovered from stock vials through culturing in YPD broth and incubated in a rotary and static platform (Incubator S80BD12; Biopar®) at 28°C for 12–24 h. The colonies observed in YPD agar were characterized by Gram staining light microscopy (1000× magnification; Light Microscope CX31; Olympus) to confirm their morphology and culture purity. In addition, *Candida parapsilosis* ATCC 22019 strain (*Candida parapsilosis* [Ashford] Langeron et Talice), described as a biofilm producer on polystyrene (Paula-Mattiello et al., 2017), was used as a reference strain for all experiments and cultured following the same protocols described for deep-sea isolates.

Biochemical tests of yeast isolates

All marine yeast isolates were pre-inoculated into YPD broth and incubated overnight at 28°C, followed by evaluation of their growth capacity in 4 ml Yeast Nitrogen Base (YNB) broth medium (HiMedia®) supplemented with different carbon sources—glucose, fructose, galactose, sucrose, maltose, xylose, arabinose, raffinose, lactose—added independently at 1% (w/v). The cultures were incubated in a rotatory platform (Orbital Shaker Incubator NT712; Nova Técnica®) for 96 h at 25°C. After incubation, isolates were classified according to their growth: absent (−), initial (+), moderate (++) and intense (+++), based on observable culture turbidity, when compared to negative control (without microorganisms). All experiments were performed in technical triplicate. Furthermore, these biochemical profiles were used as parameters to select isolates for biofilm formation assays, since a broad catabolic profile could suggest an ability to interact with complex compounds, such as polymeric structures. The selected isolates were cultured in YPD broth for taxonomic identification. To perform biofilm experiments, the same isolates were cultured in Tryptic Soy Agar (TSA) medium (1.7% casein peptone, 0.3% soya peptone, 0.5% sodium chloride, 0.25% dipotassium hydrogen phosphate, 0.25% glucose and 1.5% agar; Kasvi®) at 25°C (Incubator S80 BD12; Biopar®), until colony detection (adapted from Chari et al., 2014).

Taxonomic identification of yeast isolates

DNA extraction of selected isolates was performed using the QIAamp® DNA Stool Mini Kit (50) (Qiagen). The D1/D2 region sequence of the large ribosomal subunit rRNA (26S) gene was amplified using NL1 forward (5′-GCAT ATCAATAAGCGGAGGAAAAG-3′) and NL4 reverse (5′-GGTCCGTGTTTCAAGACGG-3′) primers through polymerase chain reaction (PCR) (Kurtzman & Robnett, 1998). Amplification was performed in a 50 µl mixture, consisting of 1.5 mmol l^{-1} $MgCl_2$, 0.2 µmol l^{-1} of each primer, 0.2 mmol l^{-1} of each dNTP, 1 U Platinum *Taq* DNA polymerase, 1X PCR reaction buffer and approximately 10 ng of genomic DNA. PCR conditions used were the following: initial denaturation at 95°C (10 min), and 27–30 cycles at 94°C (2 min), at 50°C (2 min) and at 70°C (3 min), followed by an extension at 72°C (7 min). The reaction products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega®) and sequenced by capillary method by Myleus Facility Company (Belo Horizonte). The forward and reverse sequencing reads were assembled and trimmed into single contigs using the software DNA Sequence Assembler version 5.15.0 (Phred quality score cut off of <20). The sequences produced by this study were deposited in the NCBI database as Y1 (MT151616), Y2 (MT151617), Y3 (MT151618), Y4 (MT151619) and Y5 (MT151620).

Sequences were aligned against the National Center for Biotechnology Information (NCBI) database through the online Basic Local Alignment Search Tool (BLAST) using default options (Altschul et al., 1990). Similar and reference sequences were downloaded from the NCBI database to perform phylogenetic analysis. Multiple sequence alignment followed by phylogenetic analysis was performed using tools included in Molecular Evolutionary Genetics Analysis (MEGA X; version 10.0.1) (Kumar et al., 2018). Sequences were aligned using ClustalW (Thompson et al., 1994) with default options. The resulting alignment was employed to construct a phylogenetic tree by the Maximum-Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). The statistical significance of each node in the phylogenetic tree was measured using the Bootstrap method (1000 replicates) (Felsentein, 1985).

HDPE films and accelerated weathering

HDPE pellets used in this study were produced from sugarcane biomass and donated by Indústria Petroquímica Braskem S.A. To shape HDPE films, 2 g of HDPE pellets were pressed between Teflon-coated steel plates at 130°C, applying a 3-tonne load for 2 min from a hydraulic press (MA098/C; Marconi®), obtaining large films (10–15 cm). These HDPE films were subjected to accelerated weathering. This treatment was performed using a QUV Accelerated Weathering Tester (QUV/Spray/240; Q-Lab®), which damages polymers through UV irradiation, humidity and dew (Q-Lab, 2018). Weathering conditions used in this study were based on the ASTM G154—12a protocol:

8 h at 60°C UVA radiation (340 nm UV radiation lamp with an incidence of 90°) followed by 4 h at 40°C condensation with drinking water (50% relative humidity). Accelerated weathering treatments were performed for 400, 600 and 800 h, hereafter named as 400-HDPE, 600-HDPE and 800-HDPE. For cell attachment assays, HDPE films were manually fragmented to 5 mm and selected for similar surface area and shape.

Biofilm formation assay on HDPE

HDPE films were disinfected through immersion in 70% ethanol for 30 min, followed by washing with sterile ultrapure water (Moore et al., 2001). The fragments were dried at room temperature in a laminar flow chamber (Rummel et al., 2017). For biofilm formation assays, this study employed a protocol without a biofilm induction step (adapted from Chari et al., 2014), to analyse the spontaneous biofilm formation of marine yeasts. Yeast colonies, grown in TSA medium, were used to prepare a pre-inoculum in 4 ml tryptic soy broth (TSB) medium (Kasvi®) and incubated overnight at 25°C (Orbital shaker incubator NT712; Nova Técnica®). About 100 µl of these cultures were transferred into Erlenmeyer flasks containing 10 ml TSB and incubated for 24 h at 25°C. After standardizing optical densities (OD_{600}) to 1.0 for all isolates, 1.0 µl of each monoculture was individually inoculated into 15 ml TSB in Erlenmeyer flasks containing 1 HDPE fragment. These cultures were then incubated for 24 or 48 h at 25°C, under agitation at 0.2 g. All yeast strains presented cultures with observable turbidity after 24 and 48 h incubation. All experiments were performed in technical triplicates. HDPE fragments from these cultures were employed in different analyses, as described below.

Crystal violet assay

After yeast cultivation with HDPE films, these fragments were aseptically transferred to 2 ml microtubes and rinsed with sterile ultrapure water to remove planktonic or weakly attached cells. Subsequently, 2 ml of 0.25% crystal violet (Sigma®) were added to each microtube and incubated at 25°C for 30 min and 0.2 g. HDPE films were then washed with sterile ultrapure water and dried under aseptic conditions at room temperature. The crystal violet dye bound to adhered cells was solubilized with 660 µl 70% ethanol for 30 min, at 25°C and 0.2 g. Absorbance was measured at 595 nm by SpectraMax 190 Microplate Reader (Molecular Devices®) (adapted from Chari et al., 2014).

Scanning electron microscopy

After the cell attachment assay, HDPE films were fixed with 2.5% glutaraldehyde and washed with 0.1 mol l^{-1} phosphate buffer. These fragments were dehydrated with increasing ethanol concentrations (50%–100%) and metallized with gold (BAL-TEC SCD 050–Sputter Coater; Capovani Brothers Inc®) for Scanning Electron Microscopy (SEM). The images were obtained with a secondary electron detector at 20.0 kV (INSPECT-F50; FEI Company) at the Central Laboratory of Microscopy and Microanalysis (LabCEMM) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

Atomic force microscopy

For atomic force microscopy (AFM), HDPE films from attachment assays were fixed with 2.5% glutaraldehyde, washed with sterile ultrapure water and dried at 25°C. For this analysis, a Bruker Dimension Icon PT microscope, equipped with OCR 8-10 5A probe (71 kHz resonant frequency, 0.73 N/m spring constancy, 100 µm length and 40 µm width) with a resolution of 256 × 256 pixels, was used at the LabCEMM facility (PUCRS). The height sensor mode was used to determine the average HDPE surface roughness value through NanoScope Analysis Software (version 1.50). A greater surface roughness value indicates greater heterogeneity, which is mainly characterized by the R_q parameter. R_q is given as the square root of the mean vertical deviation from a reference line (Bhushan, 2000; Rajesh Kumar & Subba Rao, 2012).

Fourier transform infrared spectroscopy

Colonized HDPE films were washed with sterile ultrapure water and dried at room temperature, and then submitted to Fourier transform infrared spectroscopy (FTIR). To collect spectra from 650 to 4000 cm^{-1} a PerkinElmer Spectrum 65 FT-IR Spectrometer with Attenuated Total Reflectance (ATR) Accessory (PerkinElmer®) was used, with resolution set at 4 cm^{-1}. The ATR diamond crystal was cleaned with acetone and a background scan was performed between each sample. A force of 30 N was used to compress samples against the diamond, to guarantee the contact between ATR crystal and sample with no damage in HDPE fragments. To identify absorption bands, a peak height algorithm of Perkin Elmer Spectrum Software was used. The resultant spectra of HDPE films were compared to absorption bands reported in the literature (Delille et al., 2007; Esmaeili et al., 2013; Hadjiev et al., 2007; Jung et al., 2018).

FTIR spectra were pre-processed on R (version 3.6.1). Baseline correction was performed through a polynomial fit using the "baseline" package, followed by vector-normalization. Non-colonized HDPE spectra were employed as negative controls, allowing for the subtraction of HDPE bands from all HDPE-biofilm spectra. For the quantification of carbohydrate and lipid proportions, the absorbance values were normalized by the respective amide II bands.

Statistical analysis

Cell adhesion data from crystal violet assay (CVA) were expressed as mean ± standard error of the mean (SEM) and normalized by the negative controls. Normality of the distributions was verified by the *Kolmogorov–Smirnov* test. Statistical differences among samples were analysed by one or two-way ANOVA followed by Tukey's test, or ANCOVA for determining the effect of weathering on yeast growth. Statistical analysis was performed using R (version 3.6.1), and a significant statistical difference was considered when $p < 0.05$.

RESULTS

Marine yeast isolates have broad catabolic profiles

We performed a growth assay by supplementing YNB broth with 1% of varied carbon sources: glucose, fructose, galactose, sucrose, maltose, xylose, arabinose, raffinose and lactose (Table 1). Our results revealed that catabolic profiles differed among isolates, with growth efficiency differences emerging after 96 h of incubation. Isolates Y8 and Y10 displayed low growth on most carbon sources. However, only Y8 did not show growth on arabinose. The wide growth range in all media indicated a wide catabolic ability for most of the marine isolates. Based on these data, and on the growth turbidity on different rich culture media used in our experiments (YPD, TSA, and TSB), five isolates (Y1 to Y5) were selected for further analysis.

Molecular identification and phylogenetic analysis

We identified the isolates by sequencing the D1/D2 region of the 26S rRNA gene and observing relatedness with phylogenetic analysis (Figure 1). All isolates were identified as *C. parapsilosis*. The phylogenetic tree showed that *C. parapsilosis* 26.2, N00-1.1 and s01-3.1 strains, which are closely related to our isolates, were collected in marine environments (unpublished data from "Research on diversity of marine yeast", College of Marine Life, Ocean University of China). Strains MROJIY04 and MF4 were isolated from plant extracts, and the JW9-2 strain from soil samples. The phylogenetic clustering indicates that our yeast isolates may share genetic features with other environmental *C. parapsilosis* strains (including marine ones) from different geographic sites. This environmental clade diverged from the reference *C. parapsilosis* ATCC 22019 strain employed as a reference on the following biofilm formation assays.

Marine yeasts show early adhesion to HDPE fragments

To determine the ability of marine isolates to colonize HDPE surfaces, we performed a CVA colorimetric

TABLE 1 Marine yeast isolates' ability to grow after 96 h at 25°C in different carbon sources

	Carbon sources							
Isolates	**Arabinose**	**Fructose**	**Galactose**	**Glucose**	**Maltose**	**Raffinose**	**Sucrose**	**Xylose**
Y1	++	+++	+++	+++	+++	+++	+++	+++
Y2	+	+++	+++	+++	+++	+++	+++	+++
Y3	++	+++	+++	+++	+++	+++	+	+++
Y4	++	+++	+++	+++	+++	+++	+++	+++
Y5	+	+++	+++	+++	++	+++	+++	++
Y6	++	+++	+++	++	++	+++	+++	++
Y7	++	+++	+++	+++	+++	+++	+++	+++
Y8	−	+	+	+	+	+++	+	+
Y9	++	+++	+++	+++	+++	+++	+++	+++
Y10	+	+	+	++	+	+	+	+

Note: Absent (−); initial (+); moderate (++); intense (+++).

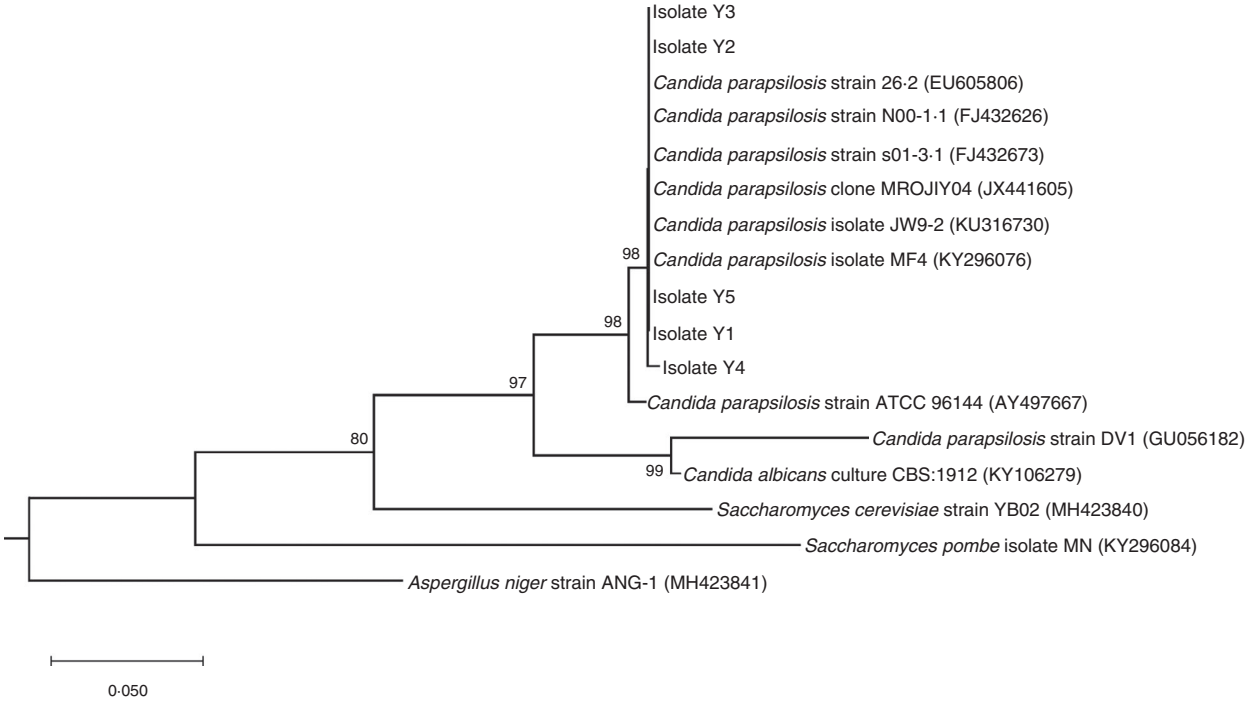

FIGURE 1 Taxonomic identification of yeast isolates Y1, Y2, Y3, Y4 and Y5. Phylogenetic analysis performed in MEGA X using large ribosomal subunit RNA (26S) reference sequences from *Candida parapsilosis* ATCC 96144 and *Candida albicans* CBS:1912 (which are curated from NCBI staff), and also sequences from other seven strains of *C. parapsilosis*, two *Saccharomyces*, and one *Aspergillus* (the latter used as an outgroup). All sequences were downloaded from the NCBI GenBank database. Phylogenetic trees were constructed by the maximum-likelihood method and Tamura-Nei model. Bootstrap percentages based on 1000 replications are shown at branch points (values below 80 were cut off)

analysis. *C. parapsilosis* ATCC 22019 strain was used as a reference strain due to its known biofilm formation ability on polystyrene, albeit under inductive protocols (Paula-Mattiello et al., 2017). The biofilm biomass was measured following 24 or 48 h of culturing to determine the most efficient biofilm-forming yeasts (Figure 2a). After 24 h of incubation on non-weathered HDPE fragments, we observed a significant difference among strains (one-way ANOVA, $F = 47.380$, $p < 0.001$). Isolates Y4 and Y5 were the most efficient biofilm producers, displaying larger biomass than the reference strain and the other marine isolates (Tukey HSD, $p < 0.01$). The optical density (OD_{600}) values for Y4 and Y5 strains cultures after 24 h and 48 h of incubation were 0.676 and 1.239, 0.737 and 1.243, respectively. After 48 h of incubation, Y3 and Y5 were the most efficient isolates (One-way ANOVA, $F = 9.723$, $p < 0.001$). Isolates Y1 and Y2 were not significantly distinct from the reference strain in either incubation interval (Tukey HSD, $p > 0.95$). Due to their higher biomass on HDPE films, isolates Y4 and Y5 were evaluated in further experiments.

To mimic abiotic degradation of HDPE, the fragments were subjected to accelerated weathering treatments, since weathering can influence the attachment of yeast cells and biofilm formation. The reference strain exhibited weak adhesion on all weathering levels and incubation intervals (Figure 2b,c). Over 24 h of incubation, isolates Y4 and Y5 exhibited decreasing attachment on weathered HDPE (ANCOVA, $F = 22.361$, $p < 0.001$), with Y5 consistently displaying larger biomass ($F = 55.541$, $p < 0.001$). The test revealed no significant interaction between strain and HDPE weathering ($F = 0.452$, $p = 0.504$), indicating that Y4 and Y5 showed a similar decrease in adhesion ability to increasingly weathered surfaces. Over 48 h of incubation, strains Y4 and Y5 showed distinct responses to HDPE weathering (ANCOVA, $F = 7.778$, $p = 0.007$), with Y5 showing a decrease in attachment due to the treatment (one-way ANOVA, $F = 10.450$, $p = 0.003$).

These marine yeast strains also showed distinct responses to incubation intervals (Figure 2d–g). Isolate Y4 exhibited a mixed response to a longer incubation, decreasing the adhesion efficiency on non-weathered HDPE (Tukey HSD, $p = 0.036$), having no response on 400-HDPE ($p = 0.999$) or 600h-HDPE ($p = 0.989$), and increasing attachment after 48 h on 800-HDPE ($p = 0.035$). On the other hand, isolate Y5 consistently showed a decrease in adhesion after 48 h of incubation, which was significant on non-weathered ($p < 0.001$) and 600-HDPE ($p < 0.001$). Taken together, these results suggest that isolate Y5 might

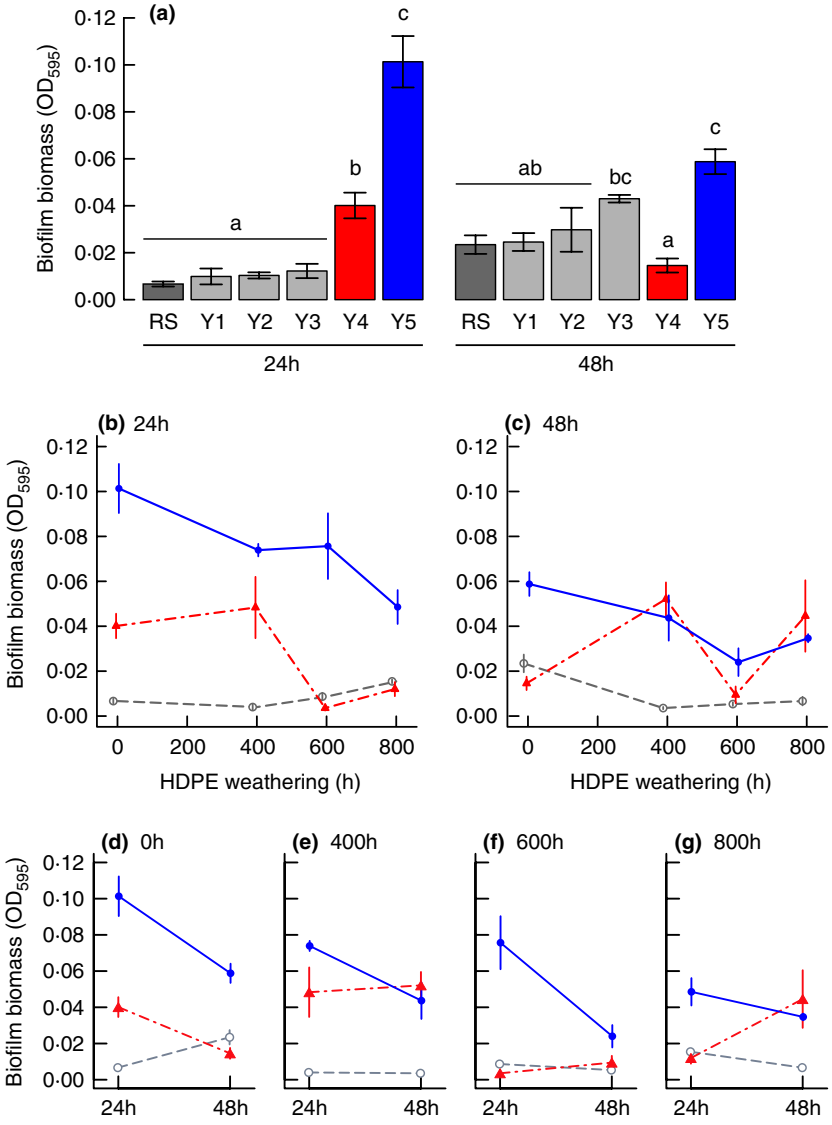

FIGURE 2 Biofilm formation on non-weathered and weathered HDPE films, quantified by CVA. (a) Biofilm formation on non-weathered HDPE of isolates Y1, Y2, Y3, Y4 and Y5 compared to reference strain (RS), after 24 and 48 h experiments. (b, c) Effect of abiotic HDPE weathering on early cell attachment of RS (-○-), Y4 (-▲-) and Y5 (—●—), as expressed by biofilm biomass. (d–g) RS (-○-), Y4 (-▲-) and Y5 (—●—) biofilm biomass comparison between 24 and 48 h of incubation for each weathering level

favour early attachment on HDPE, with higher biomass on non-weathered surfaces.

Morphological and adhesion aspects of yeast cells

To evaluate the morphological aspects of yeast-HDPE interactions, we analysed images ($n = 175$) of Y4 and Y5 isolates through SEM. To illustrate this analysis, we selected representative images of each sample (Figure 3). This visualization confirmed the early adhesion ability of marine *C. parapsilosis* Y4 and Y5 strains, mostly in the form of loose cells. Whereas both strains Y4 and Y5 showed patterns of early adhesion to HDPE surfaces, the reference strain exhibited few attached cells and pseudohyphae (800-HDPE at 24 h and 400- and 600-HDPE at 48 h). This qualitative visualization corroborates the results obtained through CVA.

Weathering and colonization increase HDPE surface heterogeneity

We performed AFM three-dimensional topography measurements to determine the surface heterogeneity as a function of weathering and yeast colonization (Table 2). Control measurements performed on non-colonized HDPE fragments indicated that the weathering treatments increased surface roughness, which was not impacted by incubation with culture media (Table 2). Colonized fragments were further analysed, and the biofilms that displayed higher heterogeneity are shown in Figure 4. By subtracting the roughness profiles of negative HDPE controls from each corresponding colonized surface, we observed that marine strains showed greater surface roughness than the reference strain (two-way ANOVA, $F = 14.665$, $p < 0.001$), which was not impacted by weathering ($F = 0.015$, $p = 0.903$). Moreover, through a paired comparison of isolates Y4 and Y5 on all treatments,

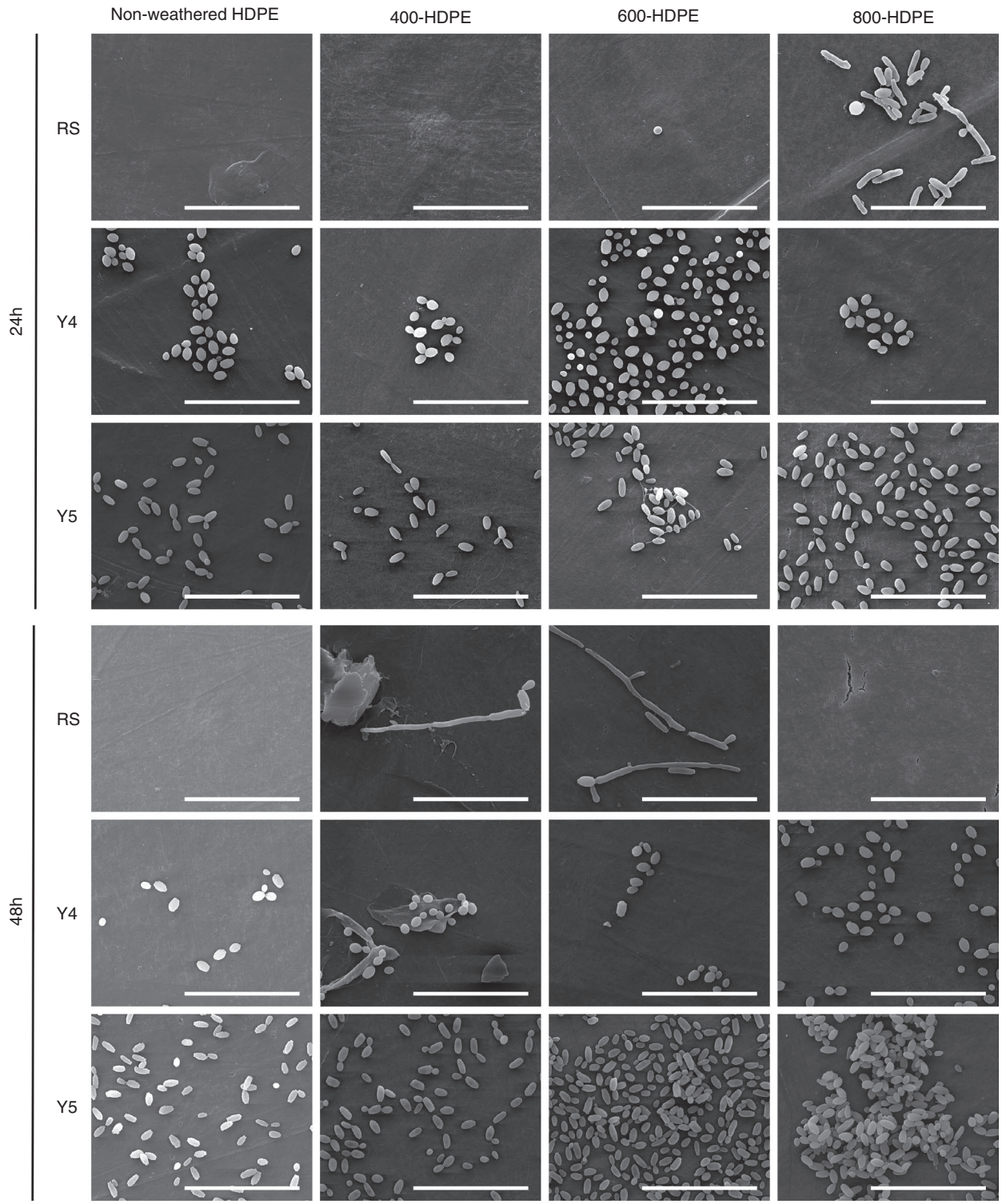

FIGURE 3 Morphological aspects of yeast-high-density polyethylene (HDPE) surface interactions. The images show early adhesion of marine isolates (Y4 and Y5) and reference strain (RS) on non-weathered and weathered (for 400, 600 and 800 h) HDPE. Magnification = 10,000×. Scale bars = 30 μm

we observed the highest roughness values on Y5 biofilms ($t = 3.489$, $p = 0.010$). This increase in surface heterogeneity is likely driven by the detection of individual yeast cells, suggesting that strain Y5 has higher attachment to HDPE surfaces.

Chemical structure of biofilms on HDPE fragments

We analysed ATR-FTIR spectra of HDPE films and HDPE-yeast samples to investigate the chemical structures of

TABLE 2 HDPE roughness values of negative control (NC), reference strain (RS) and marine isolates, obtained by AFM after biofilm formation assays

	Roughness (nm)							
	Non-weathered		400-HDPE		600-HDPE		800-HDPE	
Isolates	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
NC	30.2	30.2	76.0	76	44.6	50.9	45.6	70.9
RS	41.7	35.7	51.4	51.4	51.5	58.2	85.6	70.5
Y4	64.7	472	167	272	488	187	230	180
Y5	242	415	225	477	533	303	402	384

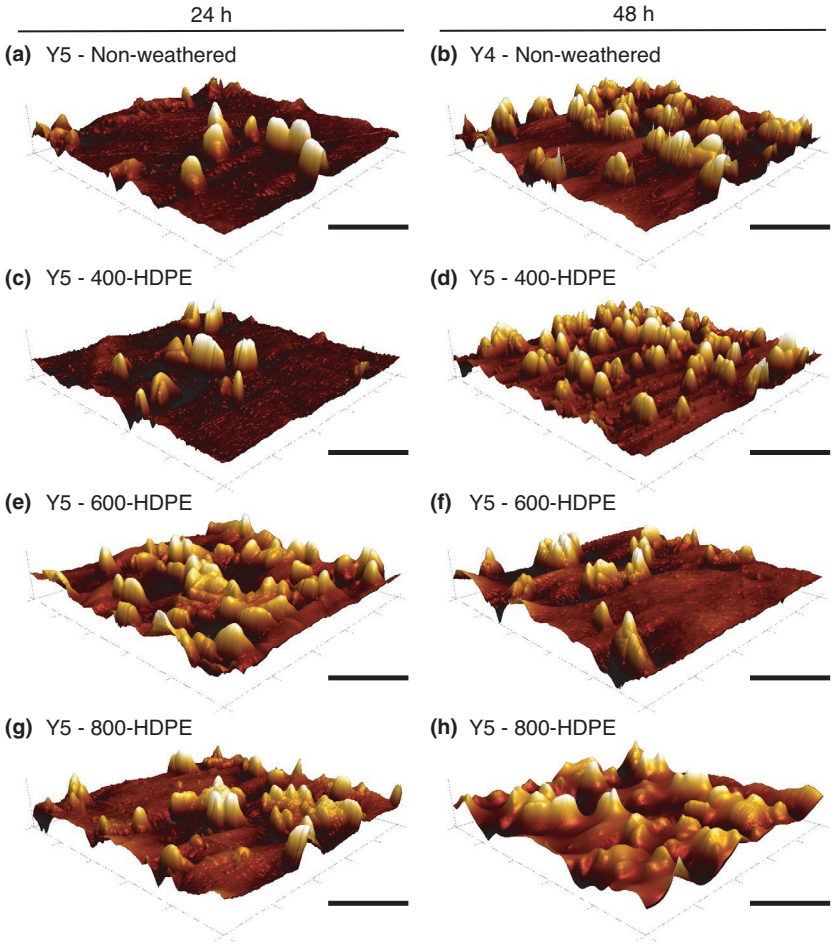

FIGURE 4 3D AFM images of marine strains biofilms that showed more heterogeneous surfaces when AFM quantitative analyses were employed after 24 and 48 h experiments. (a, b) Non-weathered HDPE: (a) Y5 and (b) Y4. (c, d) 400 h of weathering: Y5. (e, f) 600 h of weathering: Y5. (g, h) 800h of weathering: Y5. Scale bars = 10 μm

colonized fragments (Figure S1). For all raw ATR-FTIR spectra, the most prominent peaks were C-H stretch (2915 and 2847 cm^{-1}), CH_2 bend (1473 and 1451 cm^{-1}) and CH_2 rock (730 and 718 cm^{-1}), which are characteristic HDPE bands (Figure S1) (Jung et al., 2018). No shifts or significant changes in absorbance were observed for these peaks due to weathering or yeast colonization. However, new peaks appeared on weathered fragments corresponding to carboxylic acids (1735 cm^{-1}), carbonyl groups (1715 cm^{-1}), asymmetric CH_2 bend (1230 cm^{-1}) and chain breaking (1170 cm^{-1}), indicating abiotic degradation of the polymer.

Following the analysis of non-colonized HDPE, we employed ATR-FTIR to characterize the chemical structure of attached yeast cells. To isolate the properties of yeasts from the polymer background, spectra of non-colonized HDPE were subtracted from each corresponding colonized fragment. We investigated the amide II band area (1500–1600 cm^{-1}) of yeast spectra (Figure 5a,b) as a proxy for biomass (Jung et al., 2018), observing a significant difference in biomass among strains (one-way ANOVA, $F = 5.910$, $p = 0.009$). As in previous experiments, the reference strain exhibited weak growth on all treatments.

Isolate Y5 showed the highest biomass, although with inconsistent results on 24 h of incubation.

To focus on the stronger chemical signals, we narrowed analyses to isolate Y5 incubated for 48 h (Figure 5c,d). Yeast spectra were amide-normalized to show the relative proportion of carbohydrates (Figure 5c) and lipids (Figure 5d) on surfaces. While the profile of attached cells was mostly similar along weathering levels, we observed a higher proportion of both polysaccharides (900–1200 cm^{-1}) and lipids (1700–1800 cm^{-1}) on 600-HDPE. This could indicate an initial production of extracellular matrix and oily compounds, which remains to be further investigated.

DISCUSSION

Microplastic pollution has reached nearly all ecosystems on the planet, including the seafloor (Kane et al., 2020). Microbial communities present in marine sediments interact with these plastic fragments, often colonizing their surface through the formation of biofilms (Lobelle & Cunliffe, 2011; Oliveira et al., 2021). A mature biofilm can impact physical properties of the colonized polymer, altering its crystallinity, molecular weight, hydrophobicity, surface morphology, and buoyance (Luo et al., 2021). Although reports on the involvement of yeasts in such processes are scarce, marine fungi have been shown to colonize and degrade microplastics (Yuan et al., 2020), including HDPE. Nonetheless, before the development of mature biofilms and biodegradation processes take place, individual cells must efficiently attach to plastic surfaces. Here, we explored this early adhesion process by investigating interactions between yeast cells and HDPE fragments.

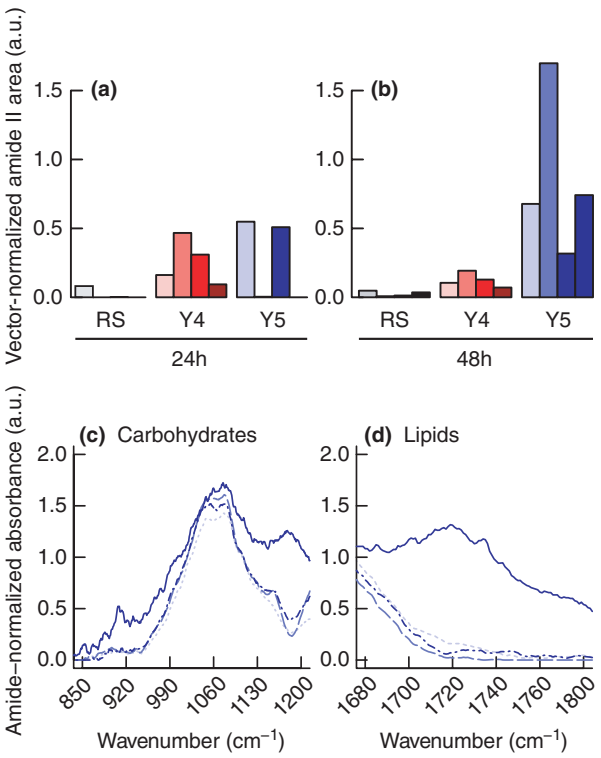

FIGURE 5 ATR-FTIR analysis of yeast-colonized HDPE fragments. (a, b) Vector-normalized amide II area (1500–1600 cm^{-1}) of the reference strain (RS), isolates Y4 and Y5, as a proxy for biofilm biomass. For each strain, the four columns indicate, from left to right, growth on non-weathered, 400-, 600-, and 800-HDPE. (c, d) Chemical characterization of Y5 biofilms incubated for 48 h on non-weathered (- - -), 400- (- - -), 600- (——), and 800-HDPE (- · -). A higher proportion of both carbohydrates (c) and lipids (d) was observed on 600-HDPE

The biochemical screening of 10 undescribed yeast isolates from deep-sea sediment, located in a recently described methane seep area (Miller et al., 2015), indicated a wide catabolic ability for these microorganisms, as previously reported for different marine yeast strains (Wang et al., 2008; Zaky et al., 2016). From these, five were selected and identified as *C. parapsilosis*, which is known as a highly adaptable yeast species occurring in natural environments (Butinar et al., 2005, 2011; Kutty & Philip, 2008; Medeiros et al., 2012; Ribas et al., 2019; Wang et al., 2008; Zaky et al., 2016). These isolates were closely related to *C. parapsilosis* marine strains obtained from GenBank, and clearly differed from the reference strain *C. parapsilosis* ATCC 22019 (from clinical origin) in both phylogenetic clustering and early adhesion characterization. Moreover, despite their phylogenetic closeness, our five marine strains clearly presented distinct abilities to colonize HDPE surfaces. We observed that strains Y4 and Y5 were efficient for early adhesion to non-weathered and weathered HDPE, whereas the reference strain displayed weak attachment on all assays. Interestingly, the reference strain has been previously reported as a biofilm producer—although on polystyrene surfaces and under inductive biofilm protocols (Paula-Mattiello et al., 2017). Since the current assays were performed without a pre-induction of biofilm formation, unlike recurring biofilm protocols (Ibarra-Trujillo et al., 2012; O'Toole et al., 2000), the present results highlight the ability of isolates Y4 and Y5 to initiate HDPE colonization.

Despite this early attachment, however, the occurrence of mature biofilms and EPS production was not observed through SEM and roughness profiles. Since the structure of biofilms changes dynamically depending on environmental conditions and growth time (Toyofuku et al., 2016), longer incubation intervals would be expected to promote the maturation of these biofilms. However, Y4 and Y5 strains showed more efficient adhesion on HPDE fragments in the first 24 h (Figure 2a), which indicates

that expanding the incubation period would not necessarily promote the development of mature biofilms in this in vitro system. Moreover, the marine strains here investigated may show higher biofilm-forming efficiency in the unstable and extreme conditions of their natural environment (e.g. high depth, low temperature and low O_2) (Miller et al., 2015).

Weathering of HDPE fragments can promote the release of additives that can be used as nutrient sources to promote microbial growth (Luo et al., 2021). However, the manner in which the microorganisms-surface interaction occurs is influenced by the size, structure, porosity and tension of the surface, as well as environmental disturbances (Yuan et al., 2020). Therefore, the decreasing cellular attachment to weathered HDPE could be explained by ultraviolet radiation treatment, which may lead to irreversible changes in polymeric chain, such as damage and exchange of chemical bonds. During the weathering process, photodegradation can modify polymer resistance, affecting its superficial layers and disrupting carbonyl groups (Nguyen et al., 2018). Moreover, this disruption of polymeric chains can reduce the density of amorphous regions, favouring abiotic oxidation processes and the appearance of shorter chains on the polymer (Gulmine et al., 2003; Nguyen et al., 2018). Thus, these irreversible changes in chemical structure caused by weathering of HPDE surfaces may interfere with the individual attachment ability of yeast strains on this polymer. Additionally, under environmental conditions, yeast strains are efficient surface colonizers and biofilm producers when interacting with other microbial species—especially bacteria, which promote physicochemical conditions for multispecies colonization (de Carvalho, 2018). Thus, despite the challenging conditions for yeast-HDPE interactions promoted by our system—composed by low initial concentrations of sole yeast isolates, tested on weathered surfaces—the marine yeast isolates here evaluated showed attachment to HDPE fragments. These data provide new perspectives regarding the early adhesion on plastic polymers for environmental isolates of *C. parapsilosis*, since most reports on yeast biofilms are related to clinical surveys (Paula-Mattiello et al., 2017; Roscini et al., 2018;).

The strains here presented were isolated from an underexplored marine ecosystem, not yet fully described in terms of their catabolic and biotechnological potential. Some microbial species may be adapted to organic contaminants that persist in the environment (Allebrandt et al., 2015; Oberbeckmann et al., 2015), exhibiting biotechnological potential for the degradation of such pollutants. Although most studies on this subject are dedicated to metabolic properties of bacterial species, certain hydrolases synthesized by fungi (*e.g.* cutinase, lipase, PETase) attract particular interest for their ability to degrade plastic polymers (Kaushal et al., 2021). Lipase and cutinase enzymes have also been reported on environmental *Candida* spp. (Carniel et al., 2017; Gautam et al., 2007; Shi et al., 2020), including *C. parapsilosis* with high levels of lipase secretion identified in the supernatant (Ribas et al., 2019). As such, the identification of marine strains of *C. parapsilosis* capable of adhering to HDPE surfaces, the first step on microbial degradation of polymers, could indicate a promising system for further studies on the biodegradation of microplastic pollutants.

Our data contribute to the characterization of catabolic properties and early adhesion on HDPE surfaces of novel *C. parapsilosis* strains from a Brazilian deep-sea ecosystem. In a broader context, the present report contributes insights to the adaptation of environmental yeast strains to recurring plastic pollutants in marine ecosystems. Future investigations should be focused on the toxicity and polymer-cell interactions of microplastics undergoing natural ageing processes, striving to understand mechanisms by which microorganisms act on the biodegradation of such polymers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Allebrandt, S.R., Napp, A.P., Mitidieri, S., Bento, F.M. & Vainstein, M.H. (2015) Bioremediation of diesel oil-contaminated soil using native hydrocarbon-degrading bacterial strains under microcosm study. *International Journal of Advanced Research Publications*, 3(11), 732–746.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410.

Bhushan, B. (2000) Surface roughness analysis and measurement techniques. In: Bhushan, B. (Ed.) *Modern tribology handbook*

volume one: principles of tribology. Boca Raton: CRC Press, pp. 49–119. Available from: https://doi.org/10.1201/9780849377877

Butinar, L., Santos, S., Spencer-Martins, I., Oren, A. & Gunde-Cimerman, N. (2005) Yeast diversity in hypersaline habitats. *FEMS Microbiology Letters*, 244(2), 229–234.

Butinar, L., Strmole, T. & Gunde-Cimerman, N. (2011) Relative incidence of ascomycetous yeasts in Arctic Coastal Environments. *Microbial Ecology*, 61(4), 832–843.

Carniel, A., Valoni, E., Nicomedes, J., Gomes, A.C. & Castro, A.M. (2017) Lipase from *Candida antarctica* (CALB) and cutinase from *Humicola insolens* act synergistically for PET hydrolysis to terephthalic acid. *Process Biochemistry*, 59, 84–90.

Chari, P.V.B., Viswadeepika, K. & Kumar, B.A. (2014) In vitro biofilm forming capacity on abiotic contact surfaces by outbreak-associated *Vibrio harveyi* strains. *Journal of Coastal Life Medicine*, 2(2), 132–140.

Coutinho, F.M.B., Mello, I.L. & de Santa Maria, L.C. (2003) Polietileno: principais tipos, propriedades e aplicações. *Polímeros*, 13(1), 01–13.

de Carvalho, C.C.C.R. (2018) Marine biofilms: a successful microbial strategy with economic implications. *Frontiers in Marine Science*, 5, 126.

Delille, A., Quilès, F. & Humbert, F. (2007) In situ monitoring of the nascent *Pseudomonas fluorescens* biofilm response to variations in the dissolved organic carbon level in low-nutrient water by attenuated total reflectance-Fourier transform infrared spectroscopy. *AEM*, 73(18), 5782–5788.

Esmaeili, A., Pourbabaee, A.A., Alikhani, H.A., Shabani, F. & Esmaeili, E. (2013) Biodegradation of low-density polyethylene (LDPE) by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in soil. *PLoS One*, 8(9), e71720.

Felsentein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39(4), 783–791.

Gadanho, M. & Sampaio, J.P. (2005) Occurrence and diversity of yeasts in the id-atlantic ridge hydrothermal fields near the Azores Archipelago. *Microbial Ecology*, 50(3), 408–417.

Gautam, R., Bassi, A.S. & Yanful, E.K. (2007) *Candida rugosa* lipase-catalyzed polyurethane degradation in aqueous medium. *Biotechnology Letters*, 29(7), 1081–1086.

Giongo, A., Haag, T., Simão, T.L.L., Medina-Silva, R., Utz, L.R.P., Bogo, M.R. et al. (2016) Discovery of a chemosynthesis-based community in the western South Atlantic Ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, 112, 45–56.

Gulmine, J.V., Janissek, P.R., Heise, H.M. & Akcelrud, L. (2003) Degradation profile of polyethylene after artificial accelerated weathering. *Polymer Degradation and Stability*, 79(3), 385–397.

Hadjiev, D., Dimitrov, D., Martinov, M. & Sire, O. (2007) Enhancement of the biofilm formation on polymeric supports by surface conditioning. *Enyzme and Microbial Technology*, 40(4), 840–848.

Ibarra-Trujillo, C., Villar-Vidal, M., Gaitán-Cepeda, L.A., Pozos-Guillen, A., Mendoza-de Elias, R. & Sánchez-Vargas, L.O. (2012) Formation and quantification assay of *Candida albicans* and *Staphylococcus aureus* mixed biofilm. *Revista Iberoamericana De micologia*, 29(4), 214–222.

Jung, M.R., Horgen, F.D., Orski, S.V., Rodriguez C., V., Beers, K.L., Balazs, G.H. et al. (2018) Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin*, 127, 704–716.

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P. et al. (2020) Seafloor microplastic hotspots controlle by deep-sea circulation. *Science*, 368(6495), 1140–1145.

Kaushal, J., Khatri, M. & Arya, S.K. (2021) Recent insight into enzymatic degradation of plastics prevalent in the environment: a mini - review. *Cleaner Engineering and Technology*, 2, 100083.

Keswani, A., Oliver, D.M., Gutierrez, T. & Quilliam, R.S. (2016) Microbial hitchhikers on marine plastic debris: human exposure risks at bathing waters and beach environments. *Marine Environment Research*, 118, 10–19.

Kettner, M.T., Rojas-Jimenez, K., Oberbeckmann, S., Labrenz, M. & Grossart, H.P. (2017) Microplastics alter composition of fungal communities in aquatic ecosystems. *Environmental Microbiology*, 19(11), 4447–4459.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549.

Kurtzman, C.P. & Robnett, C.J. (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *ALJMAO*, 73(4), 331–371.

Kutty, S.N. & Philip, R. (2008) Marine yeasts: a review. *Yeast*, 25(7), 465–483.

Lacerda, A.L.D.F., Rodrigues, L.D.S., van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R. et al. (2019) Plastics in sea surface waters around the Antarctic Peninsula. *Scientific Reports*, 9(1). Available from: https://doi.org/10.1038/s41598-019-40311-4

Lobelle, D. & Cunliffe, M. (2011) Early microbial biofilm formation on marine plastic debris. *Marine Pollution Bulletin*, 62(1), 197–200.

Lu, L., Luo, T., Zhao, Y., Cai, C., Fu, Z. & Jin, Y. (2019) Interaction between microplastics and microorganism as well as gut microbiota: a consideration on environmental animal and human health. *Science of the Total Environment*, 667, 94–100.

Luo, H., Liu, C., He, D., Xu, J., Sun, J., Li, J. et al. (2021) Environmental behaviors of microplastics in aquatic systems: a systematic review on degradation, adsorption, toxicity and biofilm under aging conditions. *Journal of Hazardous Materials*, 423, 126915.

Medeiros, A.O., Missagia, B.S., Brandão, L.R., Callisto, M., Barbosa, F.A.R. & Rosa, C.A. (2012) Water quality and diversity of yeasts from tropical lakes and rivers from the Rio Doce basin in Southeastern Brazil. *Brazilian Journal of Microbiology*, 43(4), 1582–1594.

Miller, D.J., Ketzer, J.M., Viana, A.R., Kowsmann, R.O., Freire, A.F.M., Oreiro, S.G. et al. (2015) Natural gas hydrates in the Rio Grande Cone (Brazil): a new province in the western South Atlantic. *Marine and Petroleum Geology*, 67, 187–196.

Mohsenzadeh, A., Zamani, A. & Taherzadeh, M.J. (2017) Bioethylene production from ethanol: a review and techno-economical evaluation. *ChemBioEng Reviews*, 2, 75–91.

Moore, C.J., Moore, S.L., Leecaster, M.K. & Weisberg, S.B. (2001) A comparison of plastic and plankton in the North Pacific Central Gyre. *Marine Pollution Bulletin*, 42(12), 1297–1300.

Nguyen, V.D., Hao, J. & Wang, W. (2018) Ultraviolet weathering performance of high-density polyethylene/wood-flour composites with a basalt-fiber-included shell. *Polymers*, 10(8), 12–16.

O'Toole, G., Kaplan, H.B. & Kolter, R. (2000) Biofilm formation as microbial development. *Annual Review of Microbiology*, 54(1), 49–79.

Oberbeckmann, S., Löder, M.G.J. & Labrenz, M. (2015) Marine microplastic-associated biofilms - a review. *Environmental Chemistry*, 12(5), 551–562.

Oliveira, M.M., Proenca, A.M., Moreira-Silva, E., de Castro, A.M., dos Santos, F.M., Marconatto, L. et al. (2021) Biofilms of *Pseudomonas* and *Lysinibacillus* marine strains on high-density polyethylene. *Microbial Ecology*, 81(4), 833–846.

Pathak, V.M. & Navneet (2017) Review on the current status of polymer degradation: a microbial approach. *Bioresources and Bioprocessing*, 4(1), 1–31.

Paula-Mattiello, S., de Oliveira, S.D. & Medina-Silva, R. (2017) In vitro evaluation of hydrolytic enzyme activity and biofilm formation of *Candida parapsilosis* species complex from a nosocomial environment. *Revista Da Sociedade Brasileira De Medicina Tropical*, 50(4), 558–561.

Posen, D., Griffin, W.M., Matthews, H.S. & Azevedo, I.L. (2015) Changing the renewable fuel standard to a renewable material standard: bioethylene case study. *Environmental Science and Technology*, 49(1), 93–102.

Q-Lab (2018) QUV accelerated weathering tester. [Online]. Available from: https://www.q-lab.com/products/quv-weathering-tester/quv#videos [Accessed May 2021]

Rajesh Kumar, B. & Subba Rao, T. (2012) AFM studies on surface morphology, topography and texture of nanostructured zinc aluminum oxide thin films. *Digest Journal of Nanomaterials and Biostructures*, 7(4), 1881–1889.

Ribas, R.K.D.C., Carboni, D.D.S., Cazarolli, J.C., Flôres, S.H., Ramirez-Castrillon, M. & Valente, P. (2019) Nitrogen source and pH interact and modulate lipase secretion in a non-clinical strain of *Candida parapsilosis*. *Acta Scientiarum. Biological Sciences*, 41, e45481.

Roscini, L., Vassiliou, A., Corte, L., Casagrande Pierantoni, D., Robert, V., Tascini, C. et al. (2018) Yeast biofilm as a bridge between medical and environmental microbiology across different detection techniques. *Infectious Diseases and Therapy*, 7(1), 27–34.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D. & Schmitt-Jansen, M. (2017) Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science & Technology Letters*, 4(7), 258–267.

Shi, K., Jing, J., Song, L., Su, T. & Wang, Z. (2020) Enzymatic hydrolysis of polyester: degradation of poly(ε-caprolactone) by *Candida antarctica* lipase and *Fusarium solani* cutinase. *International Journal of Biological Macromolecules*, 144, 183–189.

Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512–526.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673–4680.

Toyofuku, M., Inaba, T., Kiyokawa, T., Obana, N., Yamata, Y. & Nomura, N. (2016) Environmental factors that shape biofilm formation. *Bioscience, Biotechnology, and Biochemistry*, 80(1), 7–12.

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Van Franeker, J.A. et al. (2015) A global inventory of small floating plastic debris. *Environmental Research Letters*, 10(12), 124006.

Wang, L., Chi, Z., Yue, L., Chi, Z. & Zhang, D. (2008) Occurrence and diversity of candida genus in marine environments. *Oceanic and Coastal Sea Research*, 7(4), 416–420.

Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y. & Yu, F. (2020) Microbial degradation and other environmental aspects of microplastics/plastics. *Science of the Total Environment*, 715, 136968.

Zaky, A.S., Greetham, D., Louis, E.J., Tucker, G.A. & Du, C. (2016) A new isolation and evaluation method for marine-derived yeast spp. with potential applications in industrial biotechnology. *Journal of Microbiology and Biotechnology*, 26(11), 1891–1907.

Zettler, E.R., Mincer, T.J. & Amaral-Zettler, L.A. (2013) Life in the "Plastisphere": microbial communities on plastic marine debris. *Environmental Science & Technology*, 47(13), 7137–7146. Available from: https://doi.org/10.1021/es401288x

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