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Microplastics in the insular marine environment of the Southwest Indian Ocean carry a microbiome including antimicrobial resistant (AMR) bacteria: A case study from Reunion Island

Sababadichetty Loik [1, 2], Miltgen Guillaume [2, 3], Vincent Bryan [4], Guilhaumon François [5], Lenoble Veronique [6], Thibault Margot [1, 7, 8], Bureau Sophie [1], Tortosa Pablo [3], Bouvier Thierry [9], Jourand Philippe [5, *]

[1] Université de La Réunion, UMR ENTROPIE, 15 Avenue René Cassin, CS 92003, 97744 Saint Denis Cedex 9, La Réunion, France
[2] CHU, Laboratoire de Bactériologie, CHU Félix Guyon, Allée des Topazes, 97400 Saint-Denis, La Réunion, France
[3] Université de La Réunion, UMR PIMIT Processus Infectieux en Milieu Insulaire Tropical, CNRS 9192, INSERM 1187, IRD 249, Plateforme de recherche CYROI, 2 rue Maxime Rivière, 97490 Ste Clotilde, La Réunion, France
[4] CIRAD, UMR040 LSTM, Campus Agro Environnemental Caraïbe, BP 214-97285, Cedex 2 le Lamentin, Martinique, Antilles Françaises, France
[5] IRD, UMR ENTROPIE, 15 Avenue René Cassin, CS 92003, 97744 Saint Denis Cedex 9, La Réunion, France
[6] Université de Toulon, Aix Marseille Université, CNRS, IRD, UMR MIO, 83 Toulon, France
[7] The Ocean Cleanup, Rotterdam, the Netherlands
[8] CNRS, Université Toulouse III, Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique (IMRCP), UMR 5623, Toulouse, France
[9] UMR MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Montpellier, France

* Corresponding author : Philippe Jourand, email address : philippe.jourand@ird.fr

Abstract :

The increasing threats to ecosystems and humans from marine plastic pollution require a comprehensive assessment. We present a plastisphere case study from Reunion Island, a remote oceanic island located in the Southwest Indian Ocean, polluted by plastics. We characterized the plastic pollution on the island's coastal waters, described the associated microbiome, explored viable bacterial flora and the presence of antimicrobial resistant (AMR) bacteria. Reunion Island faces plastic pollution with up to 10,000 items/km2 in coastal water. These plastics host microbiomes dominated by Proteobacteria (80 %), including dominant genera such as Psychrobacter, Photobacterium, Pseudoalteromonas and Vibrio. Culturable microbiomes reach 107 CFU/g of microplastics, with dominance of Exiguobacterium and Pseudomonas. Plastics also carry AMR bacteria including β-lactam resistance. Thus, Southwest Indian Ocean islands are facing serious plastic pollution. This pollution requires vigilant monitoring as it harbors a plastisphere

including AMR, that threatens pristine ecosystems and potentially human health through the marine food chain.

Highlights

► Severe marine plastic pollution impacts Southwest Indian Ocean insular ecosystems. ► Plastic debris from Southwest Indian Ocean host rich microbiomes. ► Proteobacteria dominate such marine plastic microbiomes. ► These debris carry a consequent culturable bacterial flora including potential pathogens. ► AMR bacteria hitchhike on these plastics.

Keywords : Reunion Island, Plastic marine pollution, Microbiome, Proteobacteria, AMR bacteria

1. Introduction

Marine pollution by plastic wastes and debris is an important source of anthropogenic contamination in the oceans (Thushari & Senevirathna, 2020). This pollution is increasingly seen as a major concern not only for the environment, *i.e.,* contamination of pristine ecosystems with loss of biodiversity, but also for human health through contamination of marine trophic networks (Smith et al., 2018; Wright et al. 2020; Kumar et al., 2021). Plastics slowly degrade over time into smaller and smaller particles including those called "microplastics" (particle size between 0.1 - 5 mm) through weathering and physical processes as well as microbial activities (Galloway et al., 2017; Rummel et al., 2017; Jacquin et al., 2019). As a result, plastics remain present in the marine environment over very large time scales and accumulate, with an estimation of microplastic particles in 2014 reaching up to 50 trillion particles and weighing over 200,000 metric tons (van Sebille et al., 2015). Microplastic contamination of coastal and marine ecosystems reaches up to 140 particles/m^3 in water and 8766 particles/m^3 in sediments (Thushari & Senevirathna, 2020).

These microplastics are durable, often floating substrates with physical and chemical properties that can have negative impacts on entire marine ecosystems over all bathymetric zones (Rochman, 2015; Masry et al., 2021). Microplastic substrates offer new habitats and very effective dispersal ways to microbial communities that can attach through the formation of biofilms and drift along oceanic currents (Oberbeckmann & Labrenz 2020). In addition, microorganisms' communities associated with plastic, the so-called "plastisphere" (Zettler et al., 2013), have been shown to be enriched with pathogenic bacteria, including members of the genus *Vibrio* (Oberbeckmann & Labrenz 2020). Thus, pathogen-enriched floating microplastics have the potential to disperse over long distances and spread pathogenic bacteria to new marine areas and ecosystems and should therefore be considered as a threat to marine ecosystems as well as to animal and human health (Bowley et al., 2021; Stabnikova et al., 2021).

Among the pathogens underscored on microplastics, many bacterial strains have been found to harbor antimicrobial resistance (AMR) as well as resistance to heavy metals (MRG) (Bowley et al., 2021). These multidrug resistances are correlated with the presence of heavy metals, organic pollutants, and traces of antibiotics in the marine environment, which can adsorb onto plastic biofilms (Imran et al., 2019). These substances are known to promote horizontal gene transfer (HGT) of virulence and

resistance *via* mobile genetic elements (MGE) within bacterial communities (Sobecky & Hazen, 2009). Therefore, microplastics and their associated adsorbed chemicals, by promoting horizontal gene transfer in bacteria, contribute to the selection and dispersal of antimicrobial and metal resistance (Arias-Andres et al., 2018; Marathe & Bank, 2022). Finally, microplastics can have a significant impact on the spread of multidrug-resistant (MDR) pathogens, which may represent an additional threat (in terms of dangerousness compared to simple bacterial contamination) to the entire marine-related trophic network, including humans (Wright et al. 2020).

In the Indian Ocean (IO), recent reports highlight plastic accumulation along the coasts from Australia to India, in the Arabian Peninsula, along the coasts of East Africa and of the IO islands (Pattiaratchi et al., 2022). In the open ocean, the authors reported the absence of any rubbish patch in the northern IO, while a significant patch was identified in the southern IO in relation to the South Atlantic Ocean (Pattiaratchi et al., 2022) and the South Pacific Ocean (Maes et al., 2018). According to Pattiaratchi et al. (2022), Reunion Island, an oceanic island located in the southwest part of the IO, is also impacted by this marine plastic pollution. This island, located at the crossroads of southern Africa and the Indian subcontinent, is also strongly affected by the phenomenon of AMR. In the human sphere, Reunion Island is subject to a very high pressure of importation of MDR and extensively drug-resistant (XDR) pathogens, linked to the population flow in the area (Miltgen et al., 2020; Miltgen et al., 2021; Kamus et al., 2022). However, there is very little data on the environmental spread of these pathogens from human excreta after the discharge of these effluents from the wastewater treatment plants into the ocean (Miltgen et al., 2022). The same is true for microplastics that are subject to human pollution in other territories and that can drift *via* the oceans to Reunion Island.

Thus, the microbial communities existing on the plastic marine pollution that reaches the coasts of Reunion Island could be affected by this AMR phenomenon. Therefore, it is of utmost importance to determine whether the microbial communities colonizing the marine microplastic debris drifting off the coast of Reunion Island host pathogenic bacteria, potentially resistant to several antimicrobials, which should then be considered as a threat to public health.

The present study aims at *(i)* characterizing the microbiome hosted by marine microplastics drifting in the coastal waters of Reunion Island and *(ii)* addressing the presence of AMR potential

pathogens carried by these microplastics. To our best knowledge, this study is the first in the southwest Indian Ocean islands, including physico-chemical, genomic, and microbiological approaches. It integrates public health concerns and local environmental issues with the aim of shedding light on the role of microplastics and the consequences that this new human-induced niche may have, not only on the marine environment and island ecosystems, but also potentially on the entire marine food chain, up to humans, in a One Health approach (Wright et al., 2020).

2. Materials and methods

2.1 Site description and sampling methods

Reunion Island is located at 55° East 21° South, 700 km east of Madagascar (Fig. 1). The two selected sites (see map in Fig. 1) are distinct in terms of anthropogenic disturbance and oceanic influence: the first site, Livingstone (21°05'02.5"S 55°13'33.6"E), is located on the leeward west coast, at the level of the Ermitage lagoon, in Saint-Gilles municipality, while the second site *i.e.* the Tremblet beach in Saint-Philippe municipality (21°17′38″S 55°48′19″E) is located on the windward east coast. The 1st site is heavily impacted by local anthropogenic activities (Tourrand et al., 2013; Guigue et al., 2015; Lemahieu et al., 2017) while the 2nd site is a newly formed beach, almost untouched by human activity, resulting from a volcanic eruption that occurred in 2007 (Staudacher et al., 2009). The collected samples were, on one hand, the plastics from the coastal seawater (PSW) and the sand beach (PS), and on the other hand, the substrates *i.e.* the coastal sea-water (SW) and the beach sand (S). beach sand.

Plastic debris were collected from the seawater surface at 200 m from the shoreline using a manta net (mesh size: 500 µm; mouth area: 1.125 m^2) provided by the NGO "The Ocean Clean Up" (Rotterdam, The Netherlands; https://theoceancleanup.com) (Virsek et al., 2016)). The speedboat was sailing at 2 knots and the sampling time was 20 minutes, with sampling days chosen in fair weather. Three transects were set at each site to generate replicates per area (GESAMP, 2019). Between each replicate, the manta net was rinsed externally with a jet of seawater and all plastic particles were collected. Plastic concentration was calculated following Kukulka et al. (2012). Plastic debris collection number by sample according to site and substrate are reported in Supplemental Table 1. At the same time, seawater samples collected from the coastal area (3 replicates of 2 L samples per collection) were processed according to the protocol of Hinlo et al. (2017). In parallel to seawater collection, plastic

debris were collected from the beach at each site following the protocols of Besley et al. (2016) and using a 1 m × 1 m sampling quadrat. Three 50 x 10 m corridor transects running parallel to the sea were conducted for each site. We report the density of collected plastic wastes were in items/km^2. At the same time, beach sand samples were collected in triplicates according to the protocol reported by Almeida et al. (2019). From these plastic sample sorting, sub-samples of 50 microplastics particles (size < 5 mm) were randomized (Loder & Gerts, 2015) to carry out optimal DNA extraction as suggested by Debeljack et al. (2017). To avoid air contamination, the separation of the microplastics (< 5 mm) was carried out in a clean room and under a binocular magnifier under sterile conditions. These randomized subsamples were assembled in triplicates for both DNA and living microbe extractions (see further for the pre-treatment detailed protocol).

2.2. Sub-samples pre-treatments

Each microplastic sub-sample of 50 particles was treated according to the protocol of Trachoo (2004) by gentle abrasion to extract DNA and cultivable bacteria while keeping the polymer structure of the plastic intact. For this, 10 g of washed and sterile (see below) silica sand (Sigma-Aldrich, Darmstadt, Germany) was added to a sterile 50 ml Falcon tube (Fischer, Illkirch, France) containing 50 particles of microplastic. Silica sand was treated before use as follows: sand was first washed for 10 min with 2% hydrochloric acid (Merck, Darmstadt, Germany), then rinsed 3 times with MilliQ water. Silica sand was then washed for 10 minutes with sodium hypochlorite solution Emplura at final concentration of 2% (Merck, Darmstadt, Merck, Germany) and rinsed 3 times with MilliQ water. Finally, sand was rinsed once for 10 minutes with MilliQ water before being autoclaved (120°C, 20 min, 1 bar). An artificial seawater solution was reconstituted by dissolving 35 g of NaCl (Sigma-Aldrich, Darmstadt, Germany) in 1 L of MilliQ water and then autoclaved (120°C, 20 min, 1 bar). A volume of 20 mL of reconstituted sterile seawater and 20 g of sterile and washed silica sand were added to each microplastic sample. Similarly, the beach sand samples (20 g in a 50 ml Falcon tube) were supplemented with 20 ml of reconstituted sterile seawater. All samples (microplastics and beach sand) were vortexed for 60 seconds. The supernatant was collected and divided into two aliquots of 10 ml for DNA extraction and microbiological analysis. Seawater samples collected from the coastal area (3 replicates of 2 L samples

per collection) were processed according to the protocol of Hinlo et al. (2017). For each 2 L sample of sea water, there were two separate filtrations of 1 L on a sterile nitrocellulose membrane (0.22 µm): one filter was stored at -20°C for DNA extraction and the other one underwent resuspension of bacteria by vortexing in 5 ml of reconstituted sterile seawater for microbiological analyses.

2.3 DNA extractions, 16S PCR and library preparation

The 10 ml of supernatants from the plastic abrasion or sediment extractions were filtered and sterilized through 0.22 µm nitrocellulose membranes (Merck Millipore, Cork, Ireland). DNA was extracted from all nitrocellulose membranes resulting from the plastic, sediment supernatant and water filtrations, following the protocols of Debeljack et al. (2017). Briefly, the membranes were placed in Qiagen DNeasy Blood and Tissue kit columns (Qiagen GmbH, Hilden, Germany) and DNA was extracted according to the Qiagen manufacturer's instructions. After extraction, DNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific France, llkirch-Graffenstaden, France). The DNA samples were then sent to Macrogen's Next Generation Sequencing (NGS) platform (Macrogen, Seoul, Korea) for mass DNA sequencing. Library construction and sequencing were performed according to Illumina 16S metagenomic sequencing library protocols to amplify the V3 and V4 region of 16S DNA (Bukin et al., 2019). Two nanograms of genomic DNA were amplified by PCR with 5x reaction buffer, 1 mM dNTP mix, 500 nM of each of the universal F/R PCR primers and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycling condition for the 1st PCR was 3 min at 95°C, and 25 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, followed by a final extension of 5 min at 72°C. The V3-V4 domain of the 16S rDNA was amplified by PCR using the following primers V3-F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGACAGCCTACGGGNGGCWGCAG-3', V4-R: 5'-GTCTCGTGGGCTCGGAGATGTATAAGACAGGACTACHVGGGTATCTAATCC-3' (Klindworth et al., 2013) with Illumina adaptor overlays. The PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA) and 2 µl of the purified product was PCR amplified for construction of the final library containing the index using the Nextera XT index primer. The cycling condition for the 2nd PCR was the same as the 1st PCR conditions. The PCR product was purified with

AMPure beads. The final purified product was then quantified using qPCR according to the qPCR quantification protocol guide (KAPA library quantification kits for Illumina sequencing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany).

2.4 NGS analyses.

Paired sequencing (2×300 bp) was performed using the MiSeq™ platform (Illumina, San Diego, USA). Adapter pruning was performed using the fastp program, adapter sequences were removed and error correction was performed in overlapping sequences (Chen et al., 2018). The read assembly was performed by assembling pair-end sequences created by sequencing both directions of the library. The program used in this process is *FLASH (v1.2.11) (Magoc & Salzberg, 2011). Assembled reads shorter than 400 bp or longer than 500 bp were removed. Next, the preprocessing and clustering process was performed according to the protocols of Li et al. (2012): data with sequence errors were removed in order to obtain accurate OTUs. Reads containing ambiguous bases and chimeric sequences were also removed. After this process, clustering was performed based on sequence similarity with a cut-off value of 97% using CD-HIT-OTU, a comprehensive program based on cd-hit-est. Community diversity and taxonomy were analyzed according to Caporaso et al. (2010) using QIIME (v1.9.0), which is used for OTU analysis and taxonomy information. The main sequence of each OTU was referenced in the NCBI 16S database, and taxonomic information was obtained with BLASTN (v2.4.0).

2.4. Microbiological analysis

Successive serial dilutions from 1 to 10^{-2} were prepared from the bacterial supernatant resulting from the plastic / sand abrasion or the seawater 0.22 µm membrane filter resuspension using the reconstituted sterile seawater. The total bacterial flora was counted by inoculating 100 µl of these dilutions onto plate agar of Mueller Hinton + PolyViteX (PVX, BioMérieux, Marcy l'Etoile, France) and Mueller Hinton E (MHE, BioMérieux, Marcy l'Etoile, France) media as previously described (Miltgen et al., 2020). Once inoculated, the media were incubated at 35°C ± 2°C for 24 to 72 h, until the microbial colonies appeared visible. The total bacterial flora was quantified and expressed as colony forming units (CFU) per g of plastic or sand or ml of seawater. The following selective media were also

used: Columbia NaladixicAcid Agar (CNA, bioMérieux, Marcy l'Etoile, France) for the identification of the Gram-positive bacteria, Drigalski (DRIG, bioMérieux, Marcy l'Etoile, France) for Gram negative bacteria and chromID CPS ELITE (CPSE, bioMérieux, Marcy l'Etoile, France) for a control. For each sample, subcultures were analyzed, and each phenotypically distinct colony was re-isolated on Mueller Hinton agar (MHE) and incubated at 35°C ± 2°C. After 24 to 72 hours, the individual colonies were identified using MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-Of-Flight) mass spectrometry (Bizini & Greub, 2010). After identification, pathogens were counted on selective media to estimate the relative abundance of each bacterial genus. The antimicrobial susceptibility of each pathogen was assessed by the disk diffusion or gradient strips methods (Miltgen et al., 2020). After 18-24 hours, the inhibition diameter around each antibiotic disc or the MIC (minimal inhibitory concentration) were measured and the bacterial/antibiotic pair was categorized susceptible, intermediate or resistant (S/I/R) following the recommendations of The European Committee on Antimicrobial Susceptibility Testing (2020 EUCAST, https://www.eucast.org), while the resistance for isolates belonging to *Vibrio* spp. was categorized using the Clinical Laboratory Standards Institute (CLSI https://clsi.org/) recommendations.

2.5. Microplastic polymer identification

Microplastic particles were retrieved after the sand abrasion as described above. They were characterized by using Fourier Transform InfraRed spectroscopy (FTIR Nicolet i550, Thermo fisher) in ATR (Attenuated Total Reflection) mode, with wavelengths ranging from 400 to 4000 cm^{-1} (resolution of 1 cm^{-1}). The pieces were one by one pictured then pressed between diamond and base (Djaoudi et al., 2022). The diamond was cleaned between each particle analysis. Final infrared spectrums (average of 40 scans) were analyzed using SpectraGryph software and its database. Only correspondences higher than 85% were validated. The polymer IFTR identification and frequency per sample are reported in Supplemental Table 1.

2.6 Microbial community analyses based on OTUs abundance, taxonomy and statistical methods

A full overview of this analytical approach is presented in Supplemental Fig. 1. We used OTUs abundance data resulting from NGS analyses to compare microbial communities between: (i) sampling coasts (East or West coast), (ii) the matrices from which plastics were sampled (sand beach or seawater) and (iii) the sampled materials (plastic, seawater, or sand beach). The sampling DNA sequencing quality was firstly checked with rarefaction curves (Supplemental Fig. 2a, b, c, d) computed with iNEXT online (Chao et al., 2016). We used a top-down taxonomic approach to explore the differences between microbial communities by first analyzing the full data at the phylum level then focusing only on the most frequent genera belonging to the Proteobacteria phylum (genera representing more than 1% OTUs total abundance: 15 out of the 405 genera identified in the full database, Supplemental Fig. 3). OTU abundances raw data are available as Supplemental table 2a, b. The composition of microbial communities is presented using (i) barplots of the relative abundance of organisms (cumulated abundances are given in Supplemental tables 2a at phylum level and Supplemental table 3a for genera belonging to the Proteobacteria phylum level) and (ii) Non-Metric Multidimensional Scaling (NMDS) (based on Bray-Curtis dissimilarities) plots using the "vegan" package for the R software (R Core Team 2021, v4.0.4). In both graphical representations, data were grouped according to environmental parameters (combination of matrix and material), with NMDS plots showing ellipses for 95% confidence intervals. Overall differences in bacterial communities' composition were statistically assessed between sample sites, substrates and ecosystems using analysis of similarities (ANOSIM) on the same Bray-Curtis distance matrix (Dixon, 2003). Pairwise differences between groups were statistically assessed using Chi^2 tests and corrected according to the Bonferroni method. Ecological diversity was measured using several indices: specific richness and the Shannon and Simpson diversity indices. Overall differences in ecological diversity between groups were tested using non-parametric Kruskal Wallis test, followed by Duncan's post-hoc tests when more than two groups were compared and non-parametric Wilconxon Mann-Whitney test if two groups were compared. Venn diagrams were used to illustrate the dissimilarity in composition between groups (Oliveiros, 2007).

Linear models were used to compare (*i*) the abundance of plastic debris collected at the sea surface and on beaches between sites (East vs West) and polymer type and (*ii*) the abundance of

culturable bacterial populations isolated from plastic, water and sand samples from different sites or substrates. Tukey signed-rank tests were further used to evaluate pairwise differences.

3. Results

3.1. Plastic data

At the sea surface, concentrations of particles were highly variable across samples, ranging from 0 to 7,391 items per km^2 on the west coast and from 3,561 to 23,692 items per km^2 on the east coast (Table 1 and Supplemental Table 1). There was no significant difference between the west and east coasts in terms of item concentrations (p-value > 0.05) (Table 1). Most of the debris (85%) have been successfully classified by IFTR analysis. Both on the west and east coasts, the most abundant plastic polymers identified were polyethylene (PE), representing 75% and 84% of particles respectively; and polypropylene (PP) representing the remaining 25% and 16% (Table 1). There was no significant difference between the west and east coast in terms of polymer type (p.value > 0.05). For the beaches, two significant different concentrations of 0.34 ± 0.31 and 0.022 ± 0.008 item/m^2 were estimated for the west and east coast, respectively. Significant differences between the diversity of polymers on the strandings were found on the west coast, there was 50% PE, 38% PP, 9% polystyrene (PS) and 3% polyvinyl chloride (PVC) while on the east coast, there was 70% PE, 29% PP and 1% PS (Table 1).

3.2. Bacterial microbiome analyses based on the 16S rDNA sequencing.

3.2.1 Total OTUs diversity and abundance

A total of 4,052,436 reads were retained (184,201 ± 22,611 reads per sample on average) after quality filtering and chimera checking, reads abundances ranged from 138,698 to 213,256 for the plastics sampled from seawater of the West Coast (PSWw) and seawater samples from East Coast (SWe), respectively (Supplemental Tables 2a, b, c). Read numbers did not differ significantly between plastics and substrates and between coasts (Supplemental Tables 2a, b) and no clear grouping was detected when samples were compared pair wisely (Supplemental Table 2c). All rarefaction curves showed an early stationary phase indicating sufficient sequencing depth of the taxa amplified in the microplastic, sand and seawater matrices (Supplemental Fig. 2a, b, c, d). Overall, high-quality sequences were grouped into 1,084 OTUs, 877 OTUs were identified from microplastic samples, 747 from the

sand samples and 468 from seawater samples. These 1,084 OTUs (Supplemental Table 3) were tallied at an overall mean of 21,299 ± 5,127 OTUs / sample, with the difference between the East coast and West coast sites being not significant (Supplemental Table 2a, $p > 0.05$), as the difference between plastics sampled in sea water and on plastics sampled in sand (Supplemental Table 2b, $p > 0.05$). However, OTUS were significantly more abundant for plastics than for both of their substrates (seawater or sand), with OTUS being 1.46 times more abundant in plastic DNA samples than in seawater DNA samples, and 1.32 times more abundant for plastics sampled in sand (Supplemental Table 2b, $p < 0.05$). Microplastic debris from seawater shared 28.5% and 60.7% of OTUs with seawater in the western and eastern sites, respectively, while microplastics from the sandy beach shared 50.2% and 61.2% of OTUs with sand beach in the western and eastern sites, respectively (Fig. 2).

3.2.2 Diversity of OTUs at the phylum level

Phylum richness was significantly higher on sand than on water (Table 2a). However, there was no significant difference between the study sites (East versus West) nor between plastic versus non-plastic substrates (seawater or sand beach). Shannon and Simpson diversities differed significantly between study sites and between plastic and non-plastic samples (Table 2a) but not between sand and water. Shannon and Simpson diversities were higher on the east coast than on the west coast and in non-plastic samples compared to plastic samples (Table 2a). The full diversity indexes of the bacterial phylum distribution according to the site and the substrate are presented in Supplemental Table 4a.

An overall number of 19 phyla was identified in both plastics and their substrates samples (Fig. 3a, Supplemental Table 4b). All samples were dominated by Proteobacteria (75%), Bacteroidetes (11%), Cyanobacteria (5%) and Fimicutes (4%). Actinobacteria, Planctomycetes and Verrucomicrobia were also present across all the samples but at lower levels (<2%). At the phylum level, bacterial communities associated with plastics harvested in sea water (PSW) stands out from other groups (ANOSIM and Chi^2 tests, Fig. 3b and Supplemental Tables 4c and d). Height phyla were detected in seawater and sand but not on plastics: Acidobacteria, Chlamydiae, Gemmatimonadetes, Ignavibacteriae, Kiritimatiellaeota, Lentisphaerae, Spirochaetes and Thaumarchaeota. Phylum communities did not differ between sites (West *vs.* East) and matrices (samples harvested from seawater *vs.* samples harvested from the sand).

3.2.3 Proteobacteria

Proteobacteria were the dominant phylum (75.6% of the total of OTUs) and were further analyzed. To better analyze data at the genus level, we filtered out the least frequent Proteobacteria OTUs (frequency <1%). 15 out of 405 genera, representing 79.4 % of the total Proteobacteria OTUs were kept (see Supplemental Fig. 3a and Supplemental Table 3 for full results). Genera richness and Shannon diversity did not differ between sites (East *vs.* West) and environmental parameters (sand or seawater), nor between plastic and non-plastic samples (Table 2b and Supplemental Table 5a, b and c). Simpson diversity did not differ between sites (East versus West) nor between plastic versus non-plastic samples but differed significantly between substrates (sea water versus sand) ($p < 0.05$). Across all samples four genera accumulated more than 70% of the overall OTU abundance: *Psychrobacter* (21.9% in all samples and 28.8% in plastics), *Vibrio* (20.2% in all samples and 17.1% in plastics), *Pseudoalteromonas* (17.6% in all samples and 18.1% in plastics) and *Photobacterium* (14.8% in all samples and 19.4 % in plastics) were among the most found abundant genera belonging to the phylum of the Proteobacteria. Nonetheless, the composition of proteobacterial communities differed strongly between the west and the east coasts (Fig. 4a, Supplemental Table 5c). Considering plastic, seawater, and sand samples, on east coast *Vibrio* (33.1%), *Pseudoalteromonas* (25.6%) and *Photobacterium* (19.3%) were dominant whereas the genus *Psychrobacter* (39.7%) was the most abundant on the West coast. Plastic samples showed genera compositions different from seawater or sand samples (Fig. 4a, Supplemental Table 5c) with a dominance of *Psychrobacter* (28.8%), *Photobacterium* (19.5%), *Pseudoalteromonas* (18.1%) and *Vibrio* (17.1%) on plastics, and *Vibrio* (30.1%), *Pseudoalteromonas* (15.8%), *Candidatus Pelagibacter* (12.7%), *Alcanivorax* (12.6%) and *Alteromonas* (10%) in seawater and sand. Proteobacterial communities associated with microplastics or found in the water column or on the beach sand were further differentiated using NMDS ordinations and ANOSIM tests (Fig. 4b). The distribution of proteobacterial communities differed significantly (Supplemental Table 5d) between sites (green symbols VS blue symbols on Fig 4b), material collected (S + SW filled symbols versus PS + PSW empty symbols on Fig 4b), and the type of sample (combination of both site and material, represented with four ellipses on Fig 4b). However, the proteobacterial communities found on sand beaches (PS + S) versus coastal waters (PSW + SW) were similar (Fig 4b; Supplemental Table 5d).

3.3. Cultivable bacterial flora analysis and antimicrobial resistance

A dense cultivable bacterial flora was found on the microplastics from Reunion Island (3.13 x 10^7 colony forming units (CFU) /g of microplastics on average, Fig. 5, Supplemental Table 6). The total cultivable flora was significantly denser on microplastics than in seawater (4.82 x 10^2 CFU /ml of water) or on sand (3.89 x 10^4 CFU /g of sand) whatever the studied site. On the contrary, no differences were found between the density of this culturable flora on microplastics collected on the east or the west coast, or between the seawater and the sand. The fraction of potentially pathogenic bacterial microflora was isolated on selective media: a total of 105 bacterial strains could be identified at the genus level using MALDI-TOF analysis: summarized data are presented in Table 3 while full MALDI-TOF data are presented in Supplemental Table 7. Various genera belonging to the phylum Proteobacteria, Firmicutes and Bacillota were found on plastics, seawater, or sand. On plastics these culturable bacteria reach significantly higher abundances (10^3 to 10^5 CFU /g of plastic) than in seawater (1 CFU / ml of seawater) and on sand (10^2 CFU /g of sand). Noteworthy, on plastics from both sites (East or West) and both matrices (seawater or sand), the most dominant bacterial genera were *Exiguobacterium* and *Pseudomonas* with several culturable bacteria scaling from 10^3 CFU / g for *Pseudomonas* to 10^5 CFU / g for *Exiguobacterium*. Acquired resistance profiles could be sorted for 16 of the 105 strains identified by MALDI-TOF (Table 4 and Supplemental Table 8a, b). Microplastics carried bacterial strains with AMR, including strains with non-intrinsic resistances to antibiotics. The main antibiotic resistances detected concerned β-lactams such as penicillin, ampicillin and ticarcillin. On plastics, the AMR potential pathogens detected were strains belonging to *Bacillus*, *Enterococcus, Pseudomonas* and *Pantoea* genera.

4. Discussion

This study contributes to the knowledge of the health risk associated with the plastisphere in an oceanic region still very little documented.

4.1 Reunion Island suffers from plastic pollution

Our observation shows that surface coastal waters around Reunion Island are polluted by plastic. Concentrations of 10,693 ± 1,275 items/km^2 and 4,025 ± 4,760 items/km^2 were measured on the West and East coasts respectively. These results are consistent with observations in most other seas and oceans

(Thushari & Senevirathna, 2020): the Atlantic Ocean (Monteiro et al., 2018), the Artic Ocean and the North Sea (Hanninen et al., 2021; Bergman et al., 2022), the Mediterranean Sea (Cozar et al., 2014) or the subtropical gyres (Eriksen et al., 2014). This confirms that all islands scattered in the Indian Ocean are threatened by this marine pollution. Plastics have already been reported on beaches of the Maldives (Imhof et al., 2017), Madagascar (Gjerdseth, 2017), the Seychelles (Dunlop et al., 2020; Vogt-Vincent et al., 2023) or in the Chagos Islands (Hoare et al., 2022). However, the concentrations of these stranded plastics on the beaches of Reunion Island (0,35 items/m^2 on the west coast and 0.0223 items/m^2 on the east coast) are lower than reported on other Indian Ocean islands such as the Chagos Archipelago (6 items/m^2, Hoare et al,. 2022) or the Maldives (35.8 items/m^2, Imhof et al., 2017). Plastic abundance varies with environmental settings such as wind speed, swell intensity, marine currents velocity, seasonality, and the morphology of the island (Imhof et al., 2017). Reunion Island is a young volcanic island (Lenat et al., 2001) harboring few coral reefs and subject to oceanic swell and strong marine currents impacting the few beaches located on the east coast (Pous et al., 2014). Alternatively, the low plastic abundance may result from distinct oceanic influences as well as the remoteness of Reunion Island. The island is mostly under the influence of south Easterly trade winds and currents, and waters circulate from Western Australia to Reunion Island without colliding any important land mass (Schott et al., 2009). Thus, both coastal specificities and geographical isolation could limit the sedimentation of microplastics and their accumulation on the beaches of Reunion Island. Concerning the nature of the plastic polymers found in Reunion Island, whether on the sea surface or in the sand of the beaches, polyethylene and polypropylene are the most abundant polymers. The dominance of these two polymers is hegemonic across the world's seas and oceans as reported in the meta-analysis by Erni-Cassola et al. (2019).

4.2 Marine microplastic debris reaching Reunion Island host a specific microbiome

In the present study we found that a substantial proportion of up to 60% of the operational taxonomic units (OTUs) was shared between microplastics and their environment (seawater or sand beach). Seawater microplastic debris are colonized by planktonic microorganisms forming a biofilm whose composition presents expected high similarities with seawater microbiome (Zettler et al., 2013; De Tender et al., 2017). Our observation highlights the colonizing role of bacterioplankton but also

suggests a relatively new colonization (40% of OTUs are specific to plastics). Surface microplastics in the particularly turbulent coastal waters surrounding Reunion Island (strong waves and currents) are continuously mixed. This physical disturbance may prevent biofilm aging and lead to constant (re)colonization from seawater. Similarly, plastic debris reaching beaches, after being introduced into the marine environment, undergo physical and biological processes that break them down into smaller fragments, which can eventually be incorporated as part of the beach sand (Napper & Thompson, 2020). Through this process, the plastic fragments and the sandy beach substrate can contribute to each other's bacterial enrichment, which the high proportion of shared OTUs may reflect. A recent study has shown that a high proportion of OTUs shared between plastic debris and sand samples suggests that plastic debris have a significant impact on microbial communities in marine sediment (Seeley et al., 2020). Nevertheless, there is still an important proportion of OTUs (up to 38%) that are specific to the plastic debris indicating that plastics are also a microbial ecological niche for specific communities compared to the communities from the environment. A recent review suggests that such specific communities result from biofilm formation and evolution processes, with an enrichment in bacteria able to use the plastic polymer as carbon source (Sooriyakumar, et al., 2022; Zhurina et al., 2022).

4.3 Proteobacteria and Bacteroidota phyla dominate marine microplastic microbiome

Bacterial community structures differed between sample types, i.e., seawater-plastic, sand-plastic, seawater, and sand, but not between sites (East or West), suggesting a specific effect of the environment on the structure of the bacterial community. However, Proteobacteria dominated bacterial communities in all samples (75% of the total relative total abundance in average) followed by Bacteroidota (11%), regardless of the substrate (plastic, seawater, and beach sand) or the site studied (East or West coast). Proteobacteria and Bacteroidota are diverse groups of bacteria known for their ability to adapt to a wide range of environmental conditions (Kersters et al., 2006; Hahnke et al., 2016). They are commonly found in the oceans and their high abundance is well documented in seawater and marine sediments (Stal & Cretiou, 2022). Biofilm creates greater nutritional opportunities than in seawater, which may favor the development of copiotrophic bacteria such as the γ-proteobacteria (Oberbeckmann & Labrenz, 2020). In addition, plastic debris acts as a sink for pollutants in the oceans,

which can increase the concentration of pollutants in the vicinity of the plastic (Imran et al., 2019). Proteobacteria are found to tolerate high levels of pollutants and use them as an energy source, making them well adapted to live on plastic debris (Dash et al., 2013; Jacquin et al., 2019). Our observation is consistent with other reports that also observed dominance of Proteobacteria and Bacteroidota phyla associated to plastic biofilm, *e.g.* Northern Europe seas, Mediterranean Sea and the Atlantic Ocean (Oberbeckmann et al., 2014; Vaksmaa et al., 2021, Debroas et al., 2017).

4.4 Focus about the Proteobacteria microbiome

A focus among the bacterial genera belonging to the dominant Proteobacteria phylum shows noteworthy results regarding the abundance of specific dominant genera of Proteobacteria on seawater plastics, regardless of the considered coast (East or West). Thus, the genera *Photobacterium* (33%), *Pseudoalteromonas* (27%), *Psychrobacter* (18%) showed high abundances totalling up to 78% of the OTUs of Proteobacteria found on plastic. In surrounding seawater, dominant Proteobacteria (totalising more than 77% of the OTUs) were *Candidatus* (24%), *Vibrio* (24%), *Alacanivorax* (18%) and *Alteromonas* (11%). These major differences observed between the Proteobacteria found on plastics and those found in the surrounding waters argue in favor of the hypothesis that marine microorganisms have adapted to plastic as a surface for colonization (Roager & Sonnenschein, 2019). Moreover, most of these dominant genera Proteobacteria found on plastics (i.e., *Photobacterium*, *Pseudoalteromonas* and *Psychrobacter*) are known to be able to biodegrade and utilize plastics as carbon source and nutrients (Raghul et al., 2014; Muriel-Millán et al. 2021; Atanasova et al., 2021). So, their presence on plastic isolated from seawater strongly suggests that plastic debris reaching Reunion Island were probably in a degradation process due to floating for a long time from their geographical origin as suggested by Pattiaratchi et al. (2022).

Regarding the diversity of the OTUs distribution at level of the genera belonging to the Proteobacteria phylum in the plastics that reached the beach, we found that on the East coast, plastics and sand shared a similar diversity and abundance of proteobacterial OTUs, with dominant genera (accounting for more than 80% of total proteobacterial OTUs) such as *Vibrio*, *Pseudoalteromonas* and *Alteromonas*. As the formation of the East coast beach resulted from a volcanic eruption in 2007

(Staudacher et al., 2009), we can hypothesize that plastics may have transported these proteobacteria and strongly contributed to the enrichment of the sand bacterial community with these bacteria. This hypothesis is consistent with the fact that *(i)* plastic debris is known to transport and disperse microorganisms (Oberbeckman & Labrenz, 2020) and *(ii)* contributes to enrich the bacterial flora of the sediments in which it washes up (Seeley et al., 2020). In contrast, on the West coast, we found a strong difference in Proteobactreia community structure between the sand plastics, which are dominated by *Psychrobacter* (>80%), and the sandy beach where the Proteobactreia community is dominated by the genus *Dyella* (>70%). The dominance of the genus *Psychrobacter* could mean that the plastic debris reaching the west coast is enriched in polyurethane and polycaprolactone polyester (PCL), two polymers that *Psychrobacter* is specifically known to biodegrade (Atanasova et al., 2021a). Furthermore, the dominance of the genus *Dyllea* in beach sand could likely reflect residual wastewater contamination from forest or cultivated soils, as the genus *Dyllea* has been mainly isolated from crop and forest soils (Dar et al., 2020; Huang et al., 2021).

4.5 Cultivable bacteria isolated from marine microplastic debris

Total cultivable bacterial flora found on the microplastics averaged concentration of 3×10^7 colony forming units (CFU)/g of plastic, which shows that microplastics biofilms constitute an ecological niche allowing bacteria to be metabolically active and to maintain their ability to cultivate. Literature on this topic is very limited, but a recent study showed that plastic microbeads could be colonized by up to 60×10^{10} CFU/cm^2 of plastic (Çiftçi Türetken et al., 2020). Furthermore, in the present study carried out on Reunion Island, the number of culturable bacteria found on plastics were 800 times more numerous than those found in beach sediments, and up to 65×10^3 times more numerous than in coastal waters. The three-dimensional structure of biofilm produces an heterogenous physicochemical environment, resulting in an increase of possible nutritional niches and refuge from predation (Pinto et al., 2019; Zhai et al., 2023; Du et al., 2022; Zhai et al., 2023). This could explain the high microbial density and diversity observed on plastic in the present study. Similarly, culturable bacteria including potential pathogens was significantly higher on plastic debris (from 10^3 to 10^5 CFU/g plastic) than in surrounding environments (seawater or sediment) that ranged from 1 to 1000 CFU / g of

sand or ml of seawater. This is consistent with Wu et al. (2019) work that showed higher abundance of pathogenic bacterial families on microplastics, compared to substrates. The most encountered cultivable strains in our study belong to genera *Bacillus*, *Exiguobacterium* and *Pseudomonas*, which may contain pathogenic species (Bergan, 1981; Mandic-Mulec et al., 2015; Chen et al., 2017). These advise that marine plastics could pose a potential threat to human or animal health by carrying potential human or animal infectious pathogens. Noteworthy, most of these potentially pathogenic bacteria also show abilities to degrade plastics. One of the most remarkable genera is the genus *Pseudomonas* we found in high abundance (up to 10^5 CFU / g of plastic) and that showed high capacities to degrade a wide variety of plastics (Wilkes & Aristilde, 2017).

4.6 AMRs bacteria

Marine plastics have been found to enrich the plastisphere with pathogens, including genera such as *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Aeromonas* and the Enterobacterales family (Junaid et al., 2022). Most of them have acquired (non-intrinsic) resistance to antibiotics, posing a major threat to public health and the environment (Marathe & Bank, 2022). The present study is one of the first reports in the insular context of the Western Indian Ocean of the isolation of cultivable pathogens from plastics that could be evaluated for antimicrobial susceptibility testing (AST). Resistance to some of these antibiotics was found in strains of the *Bacillus*, *Enterococcus*, *Pseudomonas* and *Vibrio* genera isolated from plastics originating from seawater or sandy beaches on Reunion Island. Most of the resistance observed was directed against molecules in the β-lactam family, such as ampicillin, penicillin and ticarcillin. It is not surprising that beta-lactam resistance seems to dominate: recent studies have shown that *(i)* residual beta-lactams such as ampicillin common emerging pollutants in wastewater adsorb chemically onto plastic and thus promote resistance by enriching the selection of resistant bacteria among the plastisphere (Imran et al., 2019; Wang et al., 2021); *(ii)* beta-lactam resistance genes (*bla* genes family) are mainly supported by mobile genetic elements (MGEs), which promotes horizontal gene transfer to non-resistant bacteria within the plastisphere (Wang et al., 2021; Zhang et al., 2022c; Silva et al., 2023). Finally, these data collected in an island corresponding to “high income country” of

515 the Southwest Indian Ocean confirm that microplastic debris constitutes a reservoir of pathogens that
516 are potentially resistant to several classes of antibiotics (*i.e.,* MDR), as has already been reported
517 elsewhere in the world (Yang et al., 2019; Bowley et al., 2021; Liu et al., 2021). It is important to
518 interpret these results from a One Health perspective, as we now know that highly anthropised
519 contaminated coastal environments (wastewater treatment plant discharges in high-income countries,
520 defecation on beaches in low-income countries) can be contaminated by enteric bacteria linked to human
521 or animal excreta, and potentially MDR bacteria depending on the epidemiology of the concerned region
522 (Fernandes et al., 2020; Miltgen et al., 2022). Although in our study we did not detect any MDR bacteria
523 *per se* (Magiorakos et al., 2012), we did detect enterobacteria (*Enterobacter* sp., *Klebsiella* sp. and
524 *Pantoea* sp.,) known to be potential vectors of these *bla* genes (Poirel et al., 2002). Further studies are
525 therefore needed to investigate the microbiome of microplastics in more highly contaminated
526 environments in less developed countries at the interface with the coral reef where very typical species
527 may be encountered (*Salmonella* sp., Dr T. Bouvier, data not shown); *E.coli*, (Sellera et al., 2018) and
528 associated with potential human recontamination *via* the marine food chain or recreational use of coastal
529 marine waters. Theses enteroabacteria can also contributed to wider-scale dissemination of the *bla* beta-
530 lactam resistance genes *via* the oceanic currents. Furthermore, these results obtained in the insular
531 context of this geographical region highlight the urgent need for effective strategies to mitigate the
532 spread of antibiotic resistance associated with marine plastics to safeguard both human health and the
533 environment (Marathe & Bank, 2022; Silva et al., 2023).
534
535 5. Conclusions
536 In this study, we presented a first case of the plastisphere assessment from Reunion Island, a
537 remote oceanic island located in the Southwest Indian Ocean, polluted by plastic debris from various
538 geographical origins. The characterisation of plastic pollution in the island's coastal waters and beaches
539 indicates that Reunion Island is facing plastic pollution with up to 10,000 objects/km^2 in the coastal
540 waters, mainly consisting of polyethylene (up to 75%) and polypropylene (up to 25%). Plastic debris
541 host dense microbiomes, dominated by Proteobacteria (80%). In addition, the cultivable microbiotes
542 reached 10^9 CFU/g of microplastics, with a dominance of bacteria from genera *Exiguobacterium* (10^5

CFU/g of plastic) and *Pseudomonas* (10^3 CFU/g of plastic). This plastic debris also carries β-lactam resistant AMR bacteria such as certain strains of the genera *Bacillus*, *Enterococcus* and *Pantoea* resistant to ampicillin, penicillin and ticarcillin. Overall, our results confirm, as it has already been described for other islands in other oceans and seas, that the islands of the Indian Ocean are facing severe marine plastic pollution, the debris of which host a dense plastisphere including AMR bacteria. Our data also suggests potential risks associated with a plastic-specific microbiome for Southwest Indian Ocean socio-ecosystems.

6. The following list is the Supplemental data related to this article.

Supplemental Fig. 1: Diagram of NGS data analysis process

Supplemental Fig. 2a, b, c , d: Alpha rarefaction (Chao1) curves showing the observed sampling effort. (a) East coast Plastic Sea water (PSW) + Sea water (SW); (b) East coast Plastic Sand (PS) + Sand (S)

Supplemental Fig 3: Proteobacteria OTUs abundances according to genera. The full list of OTUs repartition according to the Proteobacteria genera including the rare Proteobacteria genera (<1%) is reported as an Excel file in Supplemental Table 3.

Supplemental Table 1: Plastic debris collection number by sample according to site and environmental parameters (seawater or sand beach) and polymer IFTR identification frequency per sample.

Supplemental Table 2a, b, c: NGS full data: number of total reads and OTUs per sample including means and standard deviation calculations. Two-way Anovas were carried out to compare OTUs abundances data by site (a) or substrate (b) or samples (c) and letters indicate significantly different means according to a test of Duncan (at $p < 0.05$). Abbreviations: S: sand; SW: sea water; PSW: plastic from sea water; PS: plastic from sand.

Supplemental Table 3: Excel file presenting the full taxonomic list of the 1084 identified OTUs and their sample distribution at Phylum level and Proteobacteria genera level.

Supplemental Table 4a: Diversity indexes of the bacterial phylum distribution according to the site and the substrate. Data are mean ± standard errors. Letters indicate significantly different means, according to a post-hoc Dunn's multiple comparison test (at $p < 0.05$).

570 Supplemental Table 4b: Total sample OTUs number and distribution per Phylum. Abbreviations: S:
571 sand; SW: sea water; PSW: plastic from sea water; PS: plastic from sand.
572 Supplemental Table 4c: Phylum OTUS distribution Chi2 test values and p.
573 Supplemental Table 4d: Analysis of similarity tests (ANOSIM) results for phylum. The groups tested
574 are i) the site (East or West), ii) the matrix (sand beach or sea water), iii) the material harvested (sand,
575 water, or plastic); and iv) the sample type (combination of site and material). The results are given as
576 ANOSIM R values and its significance.
577 Supplemental Table 5a: Diversity indexes of the Proteobacteria genus distribution according to the site
578 and the substrate. Data are means ± standard errors. Letters indicate significantly different means,
579 according to a post-hoc Dunn's multiple comparison test (at $p < 0.05$).
580 Supplemental Table 5b: Total sample OTUs number and distribution per Proteobacteria genus of which
581 frequency was >1%. Abbreviations: S: sand; SW: sea water; PSW: plastic from sea water; PS: plastic
582 from sand.
583 Supplemental Table 5c: Proteobacteria genera (15 genera > 1%) distribution Chi2 test values and p.
584 Supplemental Table 5d: Analysis of similarity tests (ANOSIM) results for Proteobacteria genera >1%.
585 The groups tested are (i) the site (East or West), (ii) the matrix (sand beach or sea water), (iii) the material
586 harvested (sand, water or plastic) and (iv) the sample type (combination of site and material). The results
587 are given as ANOSIM R values and its significance.
588 Supplemental Table 6: Total culturable bacterial flora in CFU / g or ml of substrate, Abbreviations:
589 PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach
590 sand, Values are means ±standard deviation of means (n = 3) of three independent samplings, A two-
591 anova was carried out and the different letters indicate significant differences as determined by Tukey
592 HSD test ($P \leq 0,05$).
593 Supplemental Table 7: Excel file presenting the list of the 105 cultivable bacterial strains isolated from
594 selective media and identified at genus level by MaldiTof.
595 Supplemental Table 8a, b and c: Full antibiograms data. Antibiograms were carried out according to
596 antibiotic specific spectrum related to the bacterial genus tested. From 8 to 16 ATB were tested

according to the specific ATBiograms carried out to test the bacterial strains genus. In bold: ATB resistance, in non-bold: ATB sensitivity.

Data accessibility: NGS raw data 16SrDNA sequences are deposited in zenodo data bank: https://doi.org/10.5281/zenodo.8063253

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Authors contributions : Conceptualization P.J.; Data curation B.V., F.G., L.S., P.J., M.T., V.L. and G.M.; Formal analysis B.V., L.S., P.J., M.T., V.L. and G.M; Funding acquisition: P.J and G.M.; Investigation: S.B., M.T.,V.L., L.S., and P.J. Methodology: P.J., M.G. and G.M.; Project administration: P.J.; Supervision: P.J. ; Writing - original draft : B.V., L.S. and P.J. Writing - review & editing: M.G., V.L., F.G., P.T., T.B.and G.M.

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Fig. 1. (a) Location map of Reunion island in the southwest Indian Ocean close to Madagascar and Mauritius island; (b) map of the Reunion island including the two major cities and the study site locations.

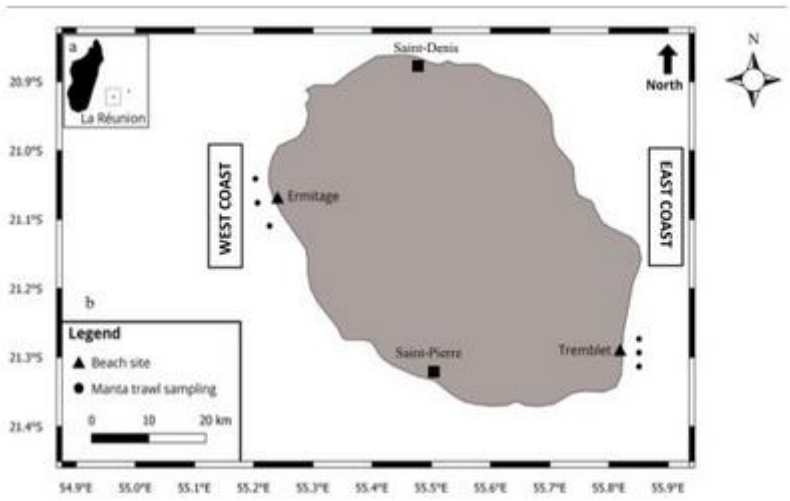

Fig. 2. Venn diagrams of the operational taxonomic unit (OTUs) distribution showing shared and specific OTUs according to site *i.e.* East coast (a) or West coast (b) in all sample types *i.e.* PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand.

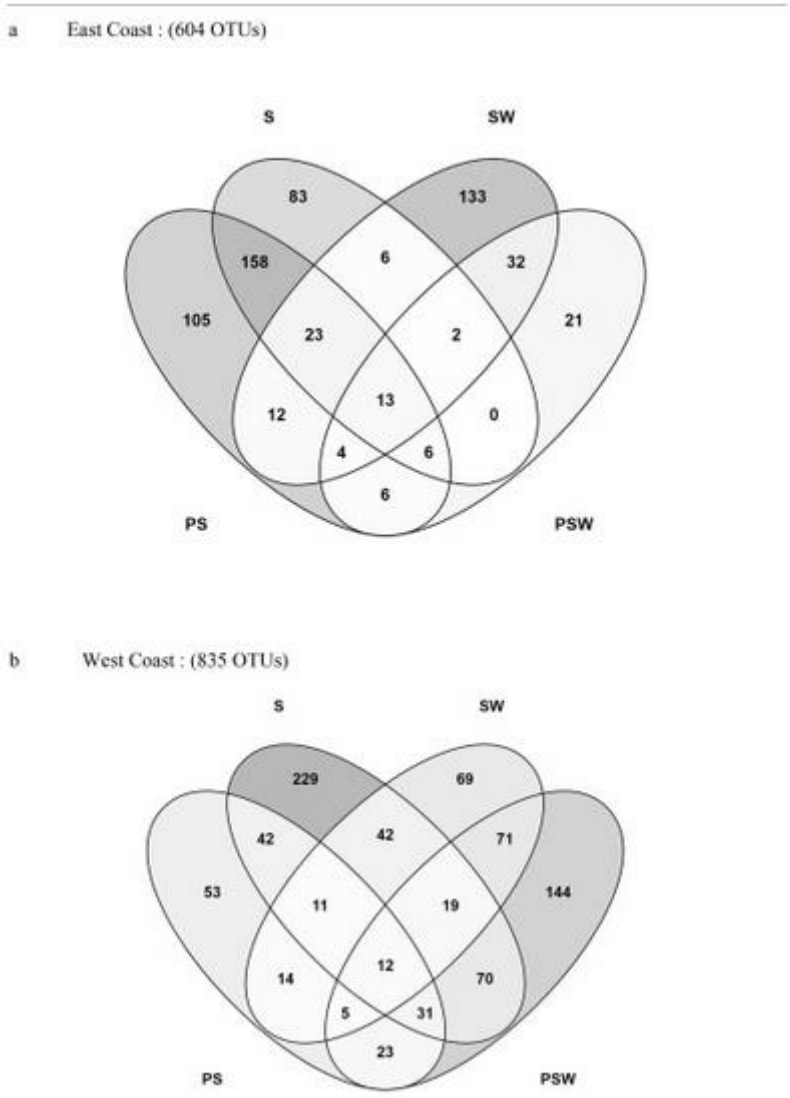

Fig. 3. OTUs distribution of the bacterial community according to the analysis at phylum level for the different substrates: PSW: plastics from coastal sea-water; SW: coastal seawater; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of bacterial phyla identified in all samples. (b) Two-dimensional NMDS ordination of bacterial community structure. Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95 %) per substrate.

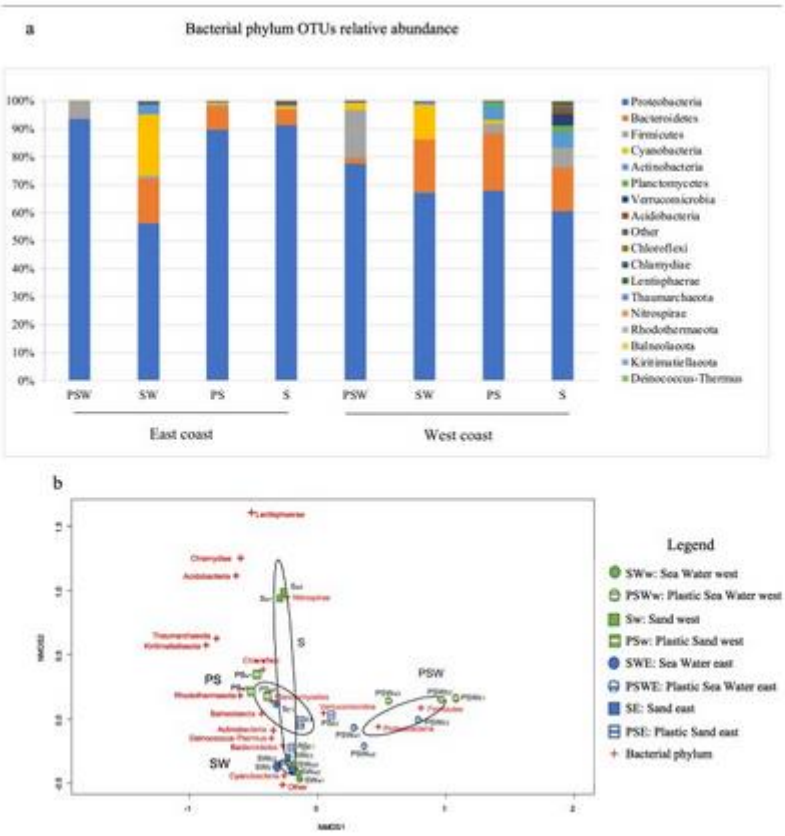

Fig. 4. OTUs distribution of the phylum of the Proteobacteria according to the analysis at genera level for the different substrates: PSW: plastics from coastal seawater; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of Proteobacteria genus identified in all samples. (b) Two-dimensional NMDS ordination of Proteobacterial community structure.
Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95 %) per substrate.

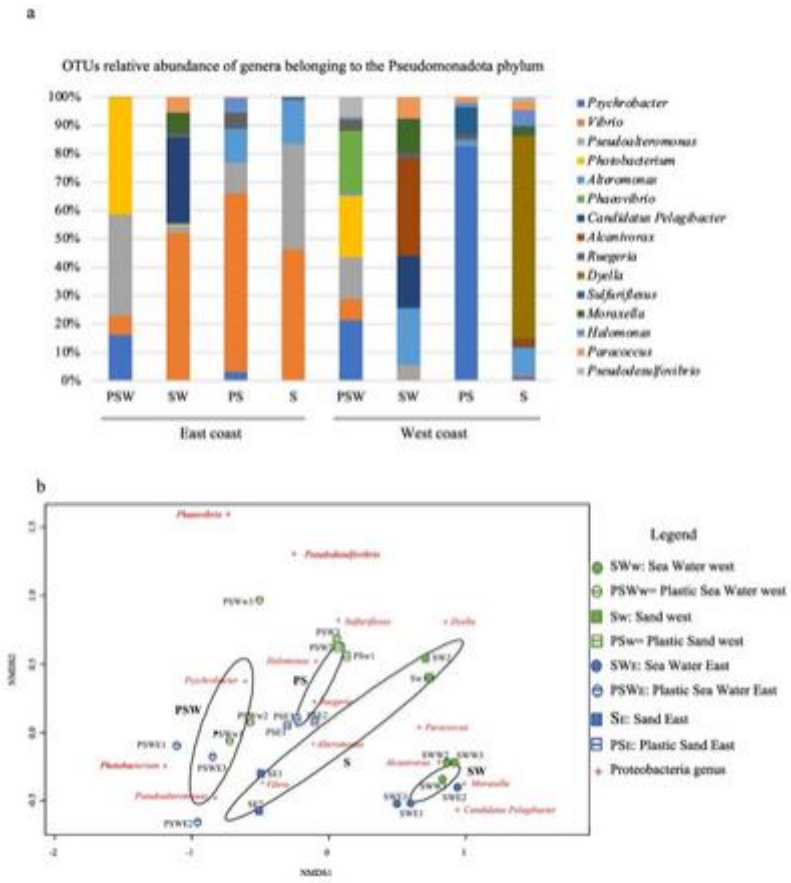

Fig. 5. Total culturable bacterial flora in CFU/g or ml of substrate. Abbreviations: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. Plots represent means, and error bars represent standard deviation of means (n = 3) of three independent samplings. The different letters above plots indicate significant differences as determined by Tukey HSD test ($p \leq 0.05$).

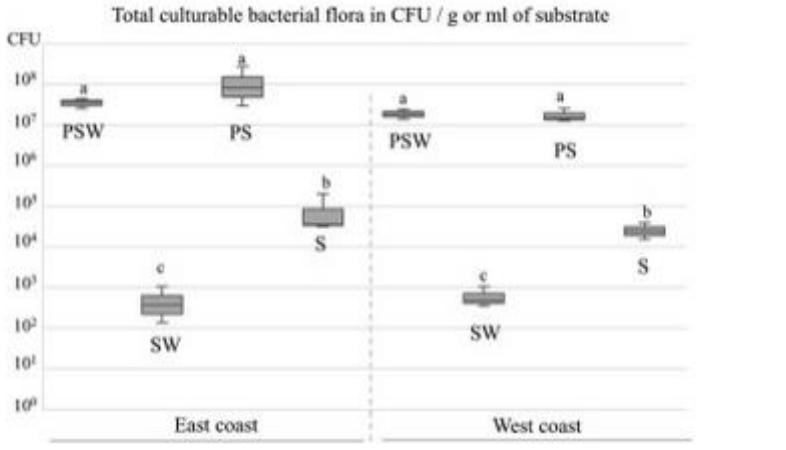

Table 1
Quantification and characterization of plastic debris collected on East coast and West coast, in seawater or on sand beach. Except for plastic polymer nature, all data are means ± stdv of samples (n = 3)/site and substrate collection.

Site		East		West	
Substrate		Seawater	Sand beach	Seawater	Sand beach
Plastic item concentration (mean ± stdv)		4025 ± 4760 items/km^2	0.022 ± 0.008 items/m^2	10,693 ± 11,275 items/km^2	0.34 ± 0.31 items/m^2
Weight of plastic item in mg/item (mean ± stdv)		1.4 ± 1.3	31.4 ± 51.2	2.6 ± 7.4	56.5 ± 43.2
Weight of microplastic in mg/sample (mean ± stdv)		24.7 ± 0.1	202 ± 90	46.7 ± 0.7	1798 ± 110
Plastic polymer (%)	Polyethylene	75	70	84	50
	Polypropylene	25	29	16	38
	Polystyrene	0	1	0	9
	Polyvinyl chloride	0	0	0	3

Table 2
Diversity indexes of the marine bacterial phyla and Proteobacteria genera found according to the combination of the site, the substrate, and the presence of plastic. Data are pooled according to three parameters: the study site (East or West), the type of substrate (sea water or sand) and the presence of plastic (plastic or non-plastic substrate i.e., seawater or sand). Data are reported as means ± standard errors. Letters indicate significantly different means according to a test of Wilcoxon–Mann–Whitney (at $p < 0.05$). Abbreviations: S: sand; SW: sea water; PSW: plastic from sea water; PS: plastic from sand. Full data of the diversity indexes per site and per substrate are presented in Supplemental Table 3.

Parameters	Groups	Richness		Shannon diversity		Simpson diversity	
		Phylum	Proteobacteria	Phylum	Proteobacteria	Phylum	Proteobacteria
Site	East (S + SW + PSW)	9.8 ± 3.1 a	8.9 ± 0.6 a	2.7 ± 0.8 a	1.05 ± 0.06 a	2.0 ± 0.5 a	0.53 ± 0.03 a
	West (S + SW + PSW)	8.4 ± 2.7 a	10.3 ± 0.4 a	1.9 ± 0.9 b	1.21 ± 0.11 a	1.6 ± 0.7 b	0.56 ± 0.06 a
Substrate	Sand (S + PS)	11.8 ± 1.8 a	10 ± 0.6 a	2.4 ± 1.1 a	1.00 ± 0.08 a	1.7 ± 0.6 a	0.48 ± 0.04 a
	Water (W + PS)	6.9 ± 1.3 b	9.3 ± 0.5 a	2.2 ± 0.8 a	1.24 ± 0.09 a	1.8 ± 0.6 a	0.61 ± 0.04 b
Plastic	Plastic (PSW + PS)	8.6 ± 2.8 a	9.7 ± 0.6 a	1.9 ± 0.6 b	1.03 ± 0.09 a	1.5 ± 0.4 b	0.51 ± 0.05 a
	Non-plastic (SW or S)	9.8 ± 3.0 a	9.5 ± 0.6 a	2.8 ± 1.0 a	1.24 ± 0.09 a	2.1 ± 0.7 a	0.60 ± 0.04 a

Table 3
Antibiotic multiresistances detected among the culturable bacterial strains. In bold are noticed non-natural antibiotic resistances. Abbreviations: PSW: plastic from sea water; PS: plastic from sand beach; SW: sea water; S: sand beach; TS: trimethoprim/sulfamethoxazole; PT: piperacilin/tazobactam; AC: amoxicillin/clavulanic acid.

Site	Substrate	Bacterial strain MT code	Bacterial genus	ATB1	ATB2	ATB3	ATB4
East	PSW	T2-14	*Pantoea*	Ampicillin	Cefadroxil	**Ticarcillin**	
		T3-42	*Pseudomonas*	TS			
	SW	T5-26	*Staphylococcus*	**Erythromycin**	**Penicillin**		
		T4-54	*Bacillus*	Amoxicillin	**Imipenem**	Penicillin G	**Vancomycin**
		T4-52	*Vibrio*	**Ampicillin**			
	PS	A1-2	*Pseudomonas*	**Ticarcillin**	TS		
		A2-15	*Bacillus*	Amoxicillin	**Imipenem**	**Penicillin G**	
		A1-13	*Vibrio*	**Ampicillin**			
	S	A4-9	*Bacillus*	Amoxicillin	**Penicillin G**		
		A6-6	*Enterobacter*	Ampicillin	AC	Cefalexin	
West	PSW	MEEP141	*Pseudomonas*	TS			
	SW	E5-28	*Pseudomonas*	TS			
		E4-13	*Bacillus*	Amoxicillin	**Clindamycin**		
	PS	B1-3	*Enterococcus*	**Rifampicin**	Vancomycin		
	S	B6-14	*Aeromonas*	**Piperacilin**	**Ticarcilin**	**Ticarcilin/Clavulanic acid**	**PT**
		B5-17	*Staphylococcus*	**Clindamycin**	**Fusidic acid**	**Penicillin**	