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The plastisphere in marine ecosystem hosts potential specific microbial degraders including *Alcanivorax borkumensis* as a key player for the low-density polyethylene degradation

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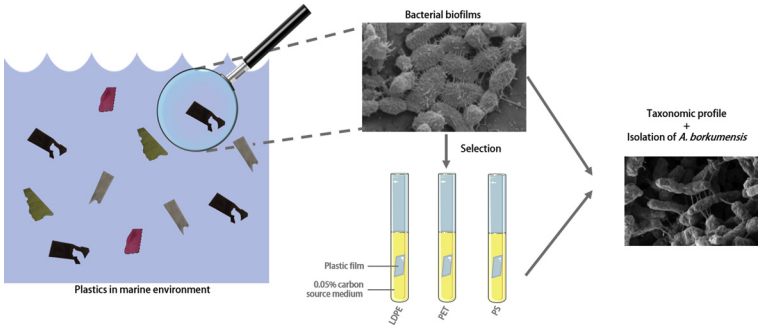
GRAPHICAL ABSTRACT



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ABSTRACT

Most plastics are released to the environment in landfills and around 32% end up in the sea, inducing large ecological and health impacts. The plastics constitute a physical substrate and potential carbon source for microorganisms. The present study compares the structures of bacterial communities from floating plastics, sediment-associated plastics and sediments from the Mediterranean Sea. The 16S rRNA microbiome profiles of surface and sediment plastic-associated microbial biofilms from the same geographic location differ significantly, with the omnipresence of *Bacteroidetes* and *Gammaproteobacteria*. Our research confirmed that plastisphere hosts microbial communities were environmental distinct niche. In parallel, this study used environmental samples to investigate the enrichment of potential plastic-degrading bacteria with Low Density PolyEthylene (LDPE), PolyEthylene Terephthalate (PET) and PolyStyrene (PS) plastics as the sole carbon source. In this context, we showed that the bacterial community composition is clearly plastic nature dependent. Hydrocarbon-degrading bacteria such as *Alcanivorax*, *Marinobacter* and *Arenibacter* genera are enriched with LDPE and PET, implying that these bacteria are potential players in plastic degradation. Finally, our data showed for the first time the ability of *Alcanivorax borkumensis* to form thick biofilms specifically on LDPE and to degrade this petroleum-based plastic.

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Table 1
Details of sampling location and number of macroplastic samples.

Type of sample	Number of samples	Abbreviations	Study
Floating plastics	7	PFS	Phylogenetic study Enrichment culture
Sedimentary plastics from Posidonia meadow	4	PH	Phylogenetic study Enrichment culture
Sediment from Posidiona meadow, 5 m depth	4	SSH	Phylogenetic study Enrichment culture
Sediment from Posidiona meadow, 10 m depth	1	SPH10	Enrichment culture
Sand from water-sediment interface	1	SSS	Enrichment culture
Sediment from Punta Bianca, 27 m depth	1	SPH27	Enrichment culture

1. Introduction

Plastic production has increased every year over the last century. Most of these synthetic polymers, i.e., 72% of the world's annually produced plastic, are released into the environment, either in landfills or in the sea (Ellen MacArthur Foundation, 2016). Plastic wastes cause serious injuries, deformities (Boren et al., 2006; Kühn et al., 2015; Alomar and Deudero, 2017; Güven et al., 2017; Poon et al., 2017) or intoxication due to the addition of endocrine disruptor compounds to the polymer matrix to enhance flexibility or color properties (Lyche et al., 2009; Rochester, 2013). The Mediterranean Sea has, on average, the highest densities of plastics in the world (Munari et al., 2017; Van der Hal et al., 2017; Galgani et al., 2000).

Low Density PolyEthylene (LDPE), PolyEthylene Terephthalate (PET), PolyStyrene (PS) are from the most widely used petro-based plastics in Europe (PlasticsEurope, 2017). The physico-chemical properties differ among these plastics, which influence their biodegradability. PET contains aromatic compounds and ester links (Yoshida et al., 2016). LDPE is composed of short branches of carbon chains and is therefore very stable (Gilan et al., 2004). Finally, PS is a styrene polymer that is durable in the environment due to its high molecular weight and very stable structure (Yang et al., 2015).

Once released in the environment, synthetic polymers are rapidly colonized by microorganisms such as fungi, diatoms or bacteria, which form a biofilm on the plastic surface. Consequently, the term "plastisphere" is used to describe the environmental niche formed by these plastics (Zettler et al., 2013). The bacterial communities of the plastisphere seemingly include bacteria capable of degrading synthetic polymers. However, the rate of degradation is very slow, and the development of plastic-associated communities is still poorly understood (Debroas et al., 2017; Bryant et al., 2016).

The bacterial composition of the plastisphere has been studied by different methods. Some studies, which have voluntarily put pieces of plastic in the environment to study the bacterial colonization of the plastisphere (De Tender et al., 2017a; Oberbeckmann et al., 2016), have shown that the compositions of these microbial communities change gradually over time. The primary colonizers of biofilms are mainly *Alphaproteobacteria* and *Gammaproteobacteria*, while members of the *Bacteroidetes* family are characteristic of the secondary colonization of plastics in the marine environment (De Tender et al., 2017a,b). In addition, the type of sample (e.g., plastic form and type of polymer), environment (e.g., port, offshore, water column or sediment), geography, season and exposure time are factors influencing biofilm formation (De Tender et al., 2017a; Oberbeckmann et al., 2016, 2014). Phylogeny-based studies have painted a picture of biofilm-associated microbial communities but lack information about their capacity for polymer degradation.

The biomineralization process of petro-sourced plastics comprises: (i) the biofilm formation on the plastic surface, (ii) depolymerization and (iii) the catabolism of the depolymerization byproducts (Artham and Doble, 2008) and (iv) biomineralization of organic matter. It has been shown that plastic-associated bacterial biofilms have a greater ability to degrade polymers than planktonic-phase bacteria (Wilkes and Aristilde, 2017). Although several studies on LDPE biodegradation with isolated bacteria or bacterial consortia have been carried out (Skariyachan et al., 2016, 2017), little is known about the biodegradation of the 3 other types of polymers. Only one bacterium, *Ideonella sakaiensis*, is known for its ability to effectively biomineralize PET (Yoshida et al., 2016).

The main objective of this work is to study the bacterial compositions of plastic-associated biofilms. For the first time, we used 16S rRNA amplicon sequencing to compare the plastic-associated microbiome compositions of macroplastics sampled from Mediterranean Sea in sediments and in floating in water from the same geographic location (Calvi, Corsica). In a second step, an enrichment method was used to select bacterial consortia capable of forming biofilms on plastics (PET, LDPE, PS). The microbial composition of the enriched biofilm that formed was assessed by denaturing gradient gel electrophoresis and 16S rRNA sequencing, and plastic degradation was assessed by scanning electron microscopy. Finally, the capacity of *Alcanivorax borkumensis*, selected on LDPE during the enrichment culture, to form a biofilm specifically on the LDPE and to degrade it is tested.

2. Materials and methods

2.1. Sampling

Macroplastics (> 5 cm) were collected in November 2016 in Northern Corsica (Calvi Bay, Mediterranean Sea) (Fig.S1; Table 1). The water temperature, salinity and pH were 20 °C, 38 g/L and 8.15, respectively. Floating plastics and sedimentary plastics from the seagrass *Posidonia oceanica* meadow were sampled in front of STARESO (42.580750 °N; 8.725783 °E). Sediments were sampled in the *Posidonia* meadow in front of STARESO (i) at 5 m depth (near the plastic samples), (ii) 10 m depth, (iii) sand from water-sediment interface and (iv) near the Punta Bianca at 27 m depth (2.569033 °N; 8.714283 °E). Plastics and sediments were immerged in sea water in 50 ml sterile tubes. All samples were stored at 4 °C during transportation. The PFS, PH and SSH samples were used for the phylogenetic study, and all the samples (PFS, PH, SSH, SPH10, SPH27 and SSS) were used for the enrichment experiment.

2.2. DNA extraction

Environmental plastic samples were scraped with a sterile scalpel blade to recover the maximum amount of bacterial biofilm. A total of 0.25 g of sediment sample was used for the DNA extraction (Supporting method 1.). Concerning the samples from the enrichment experiment, to preserve as much of the plastic surfaces as possible for further SEM analysis, the plastic associated biofilm was placed in sterile water and heated for 20 min at 60 °C. Then, 40 µl of lysozyme (100 mg/ml) and 8 µl of RNase A (10 mg/ml) were added and incubated for 20 min at 37 °C with stirring at 800 rpm. Finally, 80 µL of 20% SDS was added. The whole sample was incubated for 5 min at room temperature and

then maintained for 20 min at 60 °C. The supernatant was then added directly to the column of the Power Soil ® DNA Kit (Power Soil ® DNA Kit, QIAGEN), and DNA was purified following the manufacturer's instructions.

2.3. 16S rRNA amplicon sequencing

Amplified 460 bp amplicons of the hypervariable V3-V4 region of the 16S rRNA gene of Bacteria and Archaea (Nunes et al., 2016) were sequenced (2 × 250 bp paired-end) using high-throughput with the Illumina® MiSeq ® platform (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Sequenced samples are displayed in Table S1 (*in situ* samples) and S2 (enrichment samples). Annotation and generation of the contingency table is explained in Supporting method 2. 16S rRNA amplicon sequences were deposited at the SRA (Sequence Read Archive) in NCBI under the accession number PRJNA495136 (https://www.ncbi.nlm.nih.gov/sra/PRJNA495136).

The sequencing quality was verified with rarefaction curves using *PAST* software (Hammer et al., 2001) (Figs. S2 and S3). A-diversity indices (OTU richness and the Shannon index) were calculated on the rarefied data (5400 read counts, Limma RGui package), excluding samples with less than 5400 reads (Tables S1 and S2). Venn diagrams were realized with the *Rgui* package *limma* (Ritchie et al., 2015) to assess the OTU distributions of different conditions.

B-diversity was assessed using dissimilarity and multivariate analyses performed using the *Rgui* package *vegan* (Wang et al., 2012). To test the significance of the factors tested (Condition, place, type), PERMANOVA tests were carried out on the Bray-Curtis dissimilarity using 10,000 permutations (Table S3). A cluster dendrogram was performed using the Bray-Curtis dissimilarity with 1000 permutations with *PAST3* software (Hammer et al., 2001).

2.4. Identification and validation of response groups (RGs)

The identification and validation of Response Groups (RG) were performed as previously described (Table S4) (Nunes et al., 2016; Jacquiod et al., 2018). A total of 127 OTUs were significantly affected by the condition and plotted on a heatmap (Fig.S4). These OTUs were defined by the deviance analysis on the raw nonrarefied counts under nbGLM (negative binomial distribution and Generalized Linear Model) revised by 1000 resampling iterations of the residual variance by the utilization of the *Rgui* package *mvabund* (Dixon, 2003). Four RGs were defined with a hierarchical cluster dendrogram (Euclidean distance and average clustering) using the *Rgui* package *vegan* (Wang et al., 2012). The statistical validity of these groups was assessed by comparing the RG clustering with a null-model by Monte-Carlo simulation (Fig.S5) using all the OTUs to reinforce the randomization power.

2.5. Sample characterization (ATR-FTIR)

After DNA extraction, plastic samples were rinsed with deionized water and dried in open air. The plastic surfaces were additionally cleaned with ethanol to remove organic coatings. The type of plastic was identified using Fourier transform infrared spectroscopy (FTIR) using the attenuated total reflectance (ATR) technique (Bruker, Tensor 27) with OPUS 6.5 software (Table S5) (Mahoney et al., 2013).

2.6. Enrichment culture

Plastic samples were rinsed in sterile salt water (35 g/L of Sigma Sea Salt) to remove microorganisms that were not attached to the biofilm. Bacteria were detached from the biofilm by ultrasonic bath Transsonic 460/H (5 times of 3 min for each one) and cultured in glass tubes containing 5 ml of low carbon source marine media (0.2% ammonium sulfate, 0.05% yeast extract, 3.5% salts (Sigma Sea Salt) and 1% trace elements (0.1% $MgSO_4.7H_2O$, 0.1% $FeSO_4.7H_2O$, 0.01% $ZnSO4.7H_2O$, 0.01% $CuSO_4.5H_2O$ and 0.01% $MnSO_4.5H_2O$) in 20 mM (N-morpholino) propanesulfonic acid (MOPS) pH 8; adapted from Yoshida et al., 2016) and 2 cm^2 plastic films (600 µm thick). Three types of plastic were tested for each sample: LDPE (ExxonMobil™ LDPE, LD 150BW Wire & Cable, density: 0.923 g/cm^3, Tm: 109 °C), PS (Styrolution PS, 158 K GPPS, density: 0.1048 g/cm^3, Tm: 180–260 °C) and PET (PET BAGA 5018, Arnite A, density: 1.39 g/cm^3, Tm: 255 °C). Plastics were sterilized in 70% ethanol overnight and dried in petri dishes in sterile air. Enrichment cultures were shaken at 140 rpm at 30 °C. After 2 months of culture, biofilm formation was observed in several tubes (Table S6). The bacterial communities from these biofilms were analyzed by DGGE and 16S rRNA sequencing (as previously described 2.3.).

2.7. Denaturing gradient gel electrophoresis (DGGE)

The bacterial community structure and diversity before and after the enrichment culture were studied by DGGE (Supporting method 3). The interested bands were sequenced after amplification. PCR was performed on the sample DNA (PFS4 and PH2) using the following primers: 806R (GGACTACNNGG GTATCTAAT) and 341 F (CCTAYGG-GRBGCASCAG) (Nunes et al., 2016). The DNA was sequenced by the Sanger method (Eurofins, Cologne, Germany). The sequences were assigned to bacterial taxa with BLAST using 16S ribosomal RNA sequences (Bacteria and Archaea) database (Table S7). 16S rRNA amplicon sequences were deposited at GenBank in NCBI under the accession number MK033961:MK033964.

2.8. Network analysis

As said previously, the plastic associated communities after 2-months enrichment was sequenced and was investigated using a network analysis using OTUs whose read sum of all the samples was > 5 and that were represented in at least 2 samples (Fig. 4). Co-occurrence and exclusion links between OTUs were inferred using the nonparametric monotonic Spearmans rank correlation coefficient. Only strong and significant correlation coefficients were considered for this analysis ($|r| > .6$, FDR corrected $p < .05$, *igraph, ltm* and *psychRgui* packages). The network has been visualized and analysed using Cytoscape 3.6.1 (Shannon et al., 2003) with an organic type layout. The network discerns co-occurrent OTUs (green line) and inversely correlated OTU (red line) (Fig. 4). Network modularity have been extracted with CytoCluster using weak HC-PIN logarithm, a threshold of 2.0 and minimum size cluster of 3 (Kalenitchenko et al., 2015).

2.9. Biofilm assay and LDPE degradation by Alcanivorax borkumensis

Alcanivorax borkumensis was isolated as explained in the supporting method 4. The biofilm quantification on the sterile plastic (PVC, LDPE, PET) immerged in the medium with 0.75% hexadecane was performed with the crystal violet method (De Tender et al., 2017a; O'Toole and Kolter, 1998) and in parallel, OD at 600 nm of medium were measured to follow the bacterial growth in planktonic mode. A standard curve with the turbidity values (OD 600 nm) versus crystal violet absorbance (595 nm) was carried out in hexadecane media according to the staining procedure previously described by Mor and Sivan excepted that bacteria are pelleted by centrifugation with Eppendorf centrifuge 5818R © (10 min, 5000 rpm, room temperature) (Mor and Sivan, 2008).

For the LDPE degradation, cultures with *A. borkumensis* were started with an OD at 0.1 in medium containing 0.05% hexadecane and a sterile film (1.5 cm X 1.2 cm). The biofilm quantification and the planktonic phase were monitored each 10 days (Fig.S6). Negative control was LDPE films immersed in the medium without bacteria and undergoing exactly the same treatments as the samples. Four biological replicates were realized. After the biofilm quantification, the LDPE film

was immersed in 2% SDS (W/V) (Sodium Dodecyl Sulfate) (Mor and Sivan, 2008) overnight to detach the biofilm. Then, the film was rinsed in sterile water, dried and then weighted to calculate the percentage of weight loss.

3. Results

3.1. Plastic types of samples

Macroplastics from Corsica were collected to study plastic-associated microbiome compositions. The spectrum of each plastic type is available in the Supporting Figure File (Fig.S7). Floating plastics were identified as PolyPropylene (PP) and PolyEthylene (PE) (Table S5). Most of plastics from sediment samples were in PolyVinyl Chloride (PVC): three plastics in PVC and one in PP.

3.2. Bacterial community structure

The α-diversity of the bacterial communities of the different samples (floating plastics (PFS), plastics on sediments (PH) and sediments (SSH); Table 1) were measured via 16S rRNA amplicon sequencing showing that the plastics had much lower richness and equitability than the sediment (Fig.S8). No significant difference was observed between the floating and sedimentary plastics. The distinctness of the microbial communities on the plastic and sediment samples is visible on the Venn diagram (Fig. 1A) and nMDS analysis results (Fig. 1B). A large number of OTUs (1259) are unique to the sediment samples, representing 70.65% of sediment associated OTUs. Among the plastic specific bacteria, only 17.92% of the PH OTUs are shared with PFS community, and among the sediment specific bacteria, 24.05% PH OTUs are shared SSH samples.

The multivariate analyses (Fig. 1B) discriminated the plastic-associated communities from the sediment samples as well as the PFS communities from sedimentary plastics. The sedimentary community was mostly represented by *Proteobacteria*, especifically *Gamma-*, *Alpha-* and *Deltaproteobacteria* in comparison with the plastisphere (*p*-value: 0.020, Table S8). The plastic communities were dominated by *Bacteroidetes* contrary to sediment community (*p*-value: 1.997E-04, Table S8) and *Proteobacteria*, particularly *Gammaproteobacteria* and *Alphaproteobacteria* (Fig. 2). *Verrucomicrobia* were present in 5 plastic-attached biofilms (floating and sediment plastics) and the sediment community. *Cyanobacteria* were well represented on some floating plastics representing up to 20% of the population (see PFS 2, Fig. 2). Among floating plastics, the PFS 7 sample made of polymeric PE showed a different profile than the 3 other replicates identified as PP (Table S5). Consequently, PFS 7 was colonized by a distinct microbiome mainly represented by *Epsilonproteobacteria* (25.87%).

127 OTUs discriminate (nbGLM, p-value < 0.05) sediments, sediment-associated plastics and floating plastics and were represented on a heatmap (Fig.S4). Sediment-specific OTUs were highly represented, supporting the α- and β-diversity results. Only *Vibrio* was specific to floating plastics. Nine OTUs were enriched on sedimentary plastics: *Pseudoalteromonas,* unclassified *Flavobacteraceae* and *Gammaproteobacteria* OTUs. Finally, unclassified *Oceanospirillales*, *Glaciecola* and *Winogradskyella* genera and other unclassified bacteria were also specific to the sedimentary plastics.

3.3. Enrichment culture

In the present study, in an attempt to select bacteria that potentially degrade plastics (PET, PS, LDPE), the bacterial consortium obtained from 15 samples (PFS, PH, SSH, SSS, SPH10 and SPH27) were grown with plastic film as the main carbon source and a very low percentage of extra carbon (0.05% yeast extract) as previously used by Yoshida and colleagues who isolated *Ideonella sakaiensis* that rapidly degrade PET (Yoshida et al., 2016). After 2 months of enrichment, biofilms were visible with the naked eye on 8 LDPE, 3 PET and 1 PS films (Table S5). The biofilm community was analyzed by DGGE (Fig.S9) and 16S rRNA amplicon sequencing revealing a decrease in the richness and equitability after 2 months of culture (Fig.S10). The enrichment culture with the LDPE carbon source repeatedly showed a decrease in equitability (Fig.S11), leading to the selection of two different strains (Fig. 3A) that appeared to be *Alcanivorax borkumensis* (band a) and *Microbulbifer* sp. (band b). *Alcanivorax borkumensis* represented less than 0.2% of the bacterial community from Corsica plastics and more than 60% of the bacterial community after 2 months of culture in plastic-associated

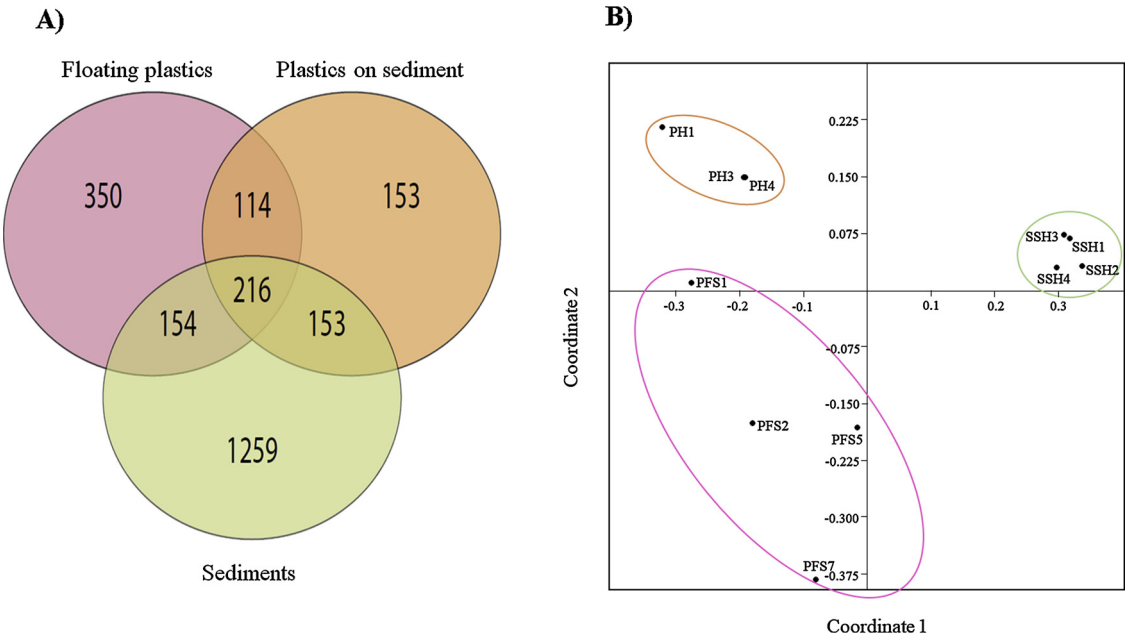

Fig. 1. (A) Venn diagram showing overlap of bacterial OTUs for the pooled floating plastics, plastics on sediments and sediments. Unique OTUs or shared OTUs are represented by numbers inside the circles for a given sample type. (B) Non-metric multidimensional scaling (nMDS) profile of the pairwise community dissimilarity (Bray-Curtis) indices of the 16S sequencing data for floating plastics (PFS), plastics on sediments (PH) and sediments (SSH) from Corsica. The three types of samples are well separated.

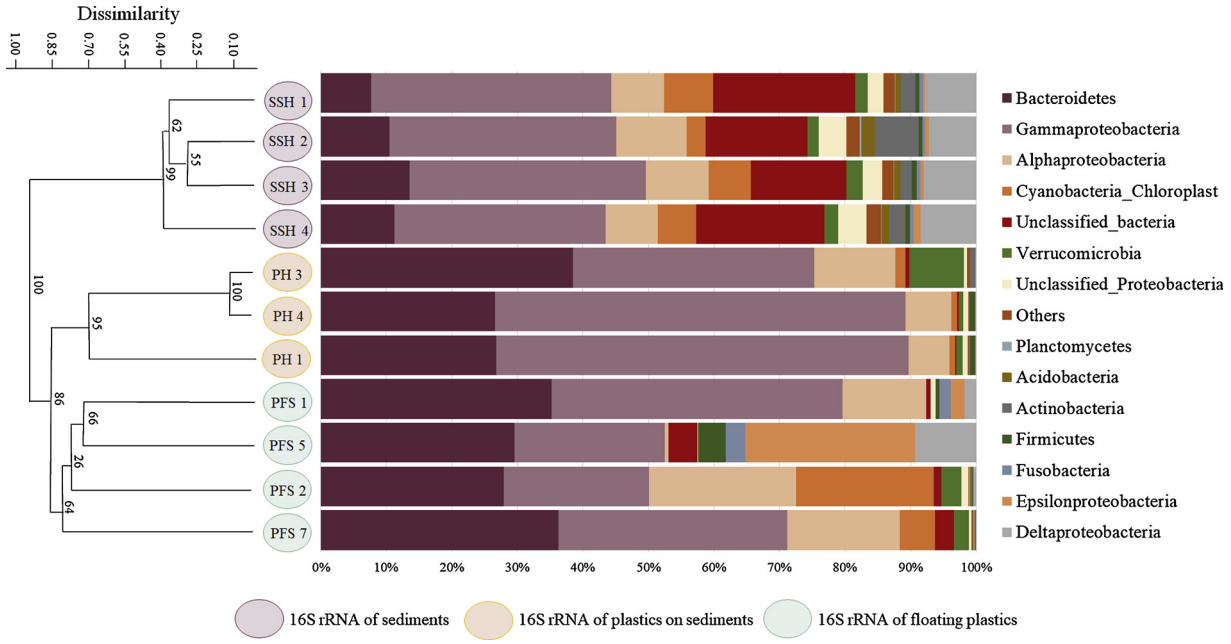

Fig. 2. Cluster dendrogram analysis (correlation index = 0.957) of the phylogenetic profiles based on the Bray-Curtis dissimilarity index assessed by 1000 bootstrap replicates on 16S rRNA amplicon sequencing.

biofilm (Fig.S12). Concerning *Microbulbifer* sp., this bacterium represented 96% of the microbial population after enrichment on LDPE.

The family composition of the biofilm after enrichment was studied in more detail (Fig. 3B). On 5 LDPE films (PFS1, PFS2, PFS4, PFS5 and PH4), the most abundant family was *Alcanivoracaceae*, as shown before with the enrichment of *Alcanivorax borkumensis*. This family was not represented on the other plastic films, making this bacterium a specialist for LDPE. The other two families most represented in these samples were *Rhodobacteraceae* and *Flavobacteriaceae*. The *Rhodobacteraceae* family was also very abundant in the biofilm on the PET and was composed of the same genera (i.e., *Ruegeria, Sulfitobacter, Roseovarius* and *Loktanella*) in both the LDPE and PET films. *Arenibacter* was also present on one PET sample. Finally, on the PS biofilm, 2 other genera represented the *Flavobacteriaceae* family: *Winogradskyella* and *Salinimicrobium*. The "PH2" and "PFS7" LDPE samples were dominated by the *Alteromonadaceae* family; *Microbulbifer* represented 96% of the "PH2" sample community, and *Marinobacter* represented 37% of the "PFS7" community. *Vibrionaceae* were very enriched (up to 30%) in several samples (PFS7 LDPE, PFS7 PS, PFS6 PET, SSH PET and SSS PET) and were represented by two genera, namely, *Vibrio* and *Photobacterium. Desulfovibrionaceae* was represented by only one genus, *Desulfovibrio*. *Pseudomonadaceae* represented 20% of the bacterial community of biofilms on PS, with *Pseudomonas* as the only representative genus. On the PFS6 PET sample, *Marinomonas*, part of the *Oceanospirillaceae* family, composed approximately 10% of the microbial community. Finally, *Bacillus* was present on SSS PET.

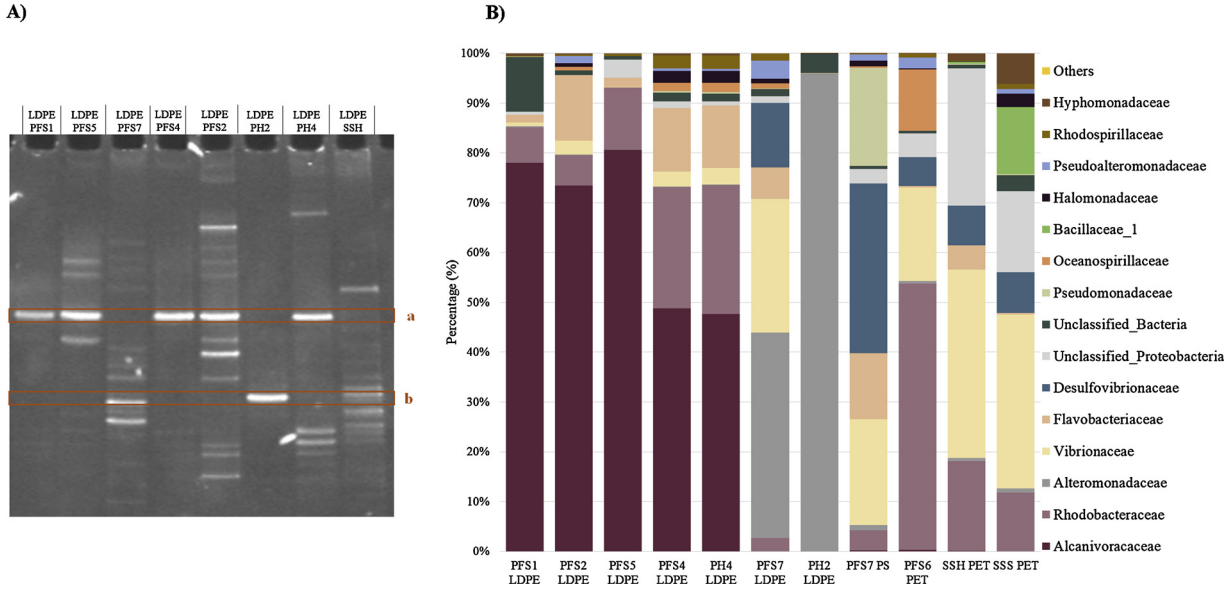

Fig. 3. Two months of enrichment culture analysed with (A) DGGE analysis of PCR-amplified 16S rRNA gene fragments of the bacterial community on LDPE after. The orange boxes highlight the 2 bands (a and b) that are highly enriched in the different biofilms on the LDPE. The first band "a" corresponds to *Alcanivorax borkumensis,* and the second band "b" corresponds to *Microbulbifer* sp and (B)Abundance of bacterial families present in biofilms on LDPE, PET and PS obtained from 16S rRNA amplicon sequencing of direct DNA extract.

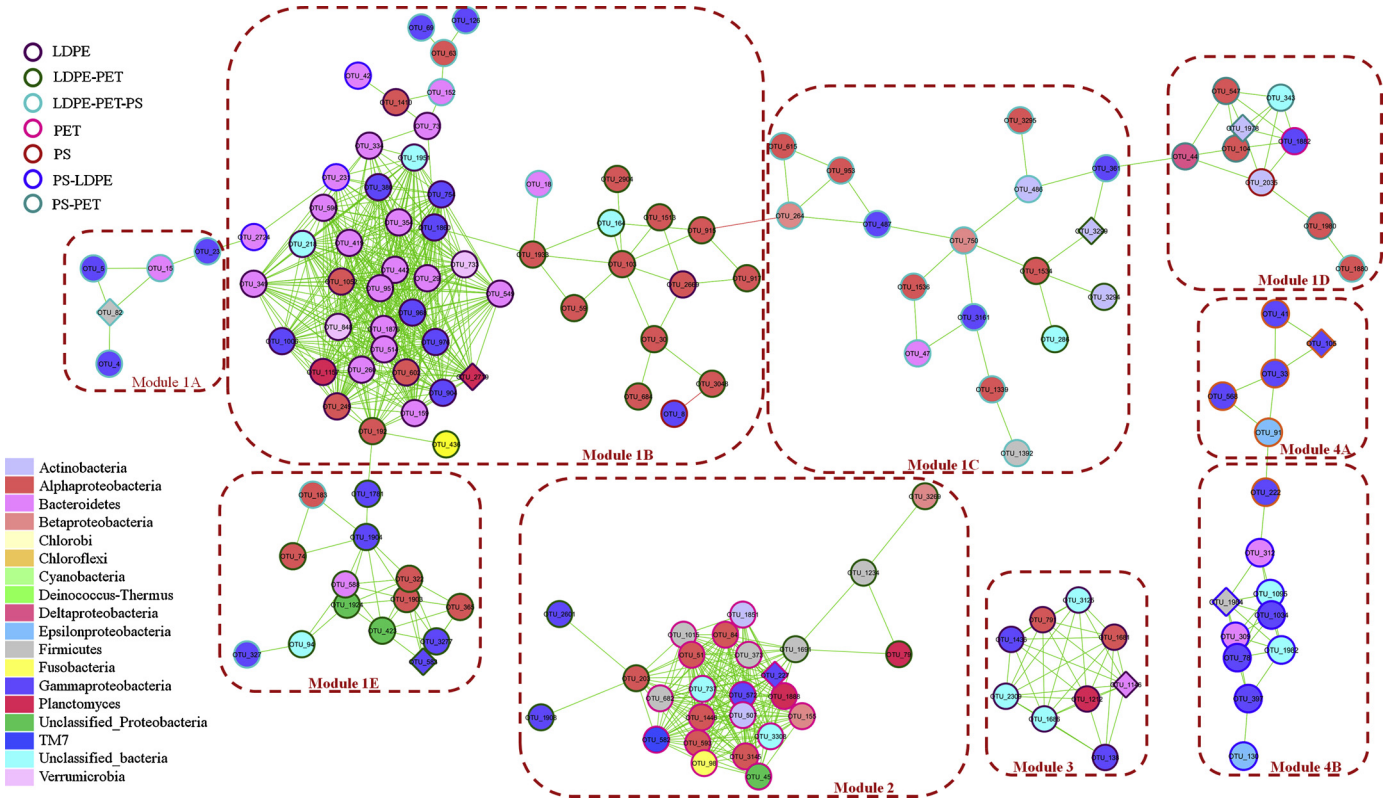

Fig. 4. Network diagram (Spearman correlation, significantly correlated (FDR corrected p-value < 0.05) of OTUs after the 2 months of enrichment obtained from 16S rRNA amplicon sequencing. Modules were defined (HC-PIN algorithm) and the seed of these are represented by squares. Green lines represent co-occurrent OTUs and red lines those that are counter-occurrent. The composition of each module is Table S9 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

A network analysis was performed on the plastic-associated microbiome after an enrichment with plastic as main carbon source (Fig. 4) to light co-occurrence of species through the entire set of plastics. Modules 1B, 2 and 3 display highly connected OTUs. Respectively 439, 201 and 33 internal links were identified within the module (Table S9). The module 1B was mainly composed of OTUs strictly associated with LDPE plastic, including a large number of OTUs that may be involved in hydrocarbon degradation such as, *Rhodobacteraceae*, *Flavobacteriales*, *Gramella*, *Oleibacter*, *Rhizobiales*, *Halomonas* and *Alcanivorax* that contain species involved in petroleum aliphatic hydrocarbon degradation (Teramoto et al., 2011; Prince et al., 2010). *In situ*, the *Flavobacteraceae* and *Rhodobacteraceae* were also dominant in plastisphere (Fig. 5). This module was clearly separated from the module 1C, mainly containing OTUs that were present on the three types of plastic without any known involvement in hydrocarbon degradation. Bacteria selected on PET and PS, plastics containing a cycle in their structure, were found in the module 1D. The module 3, containing exclusively OTUs present on LDPE, was also composed of hydrocarbon degradation bacteria such as *Alcanivorax* and *Rhodobacteraceae* (Prince et al., 2010). Most OTUs composing module 2 were specific to PET. Among them, *Clostridiales*, *Cellulosimicrobium*, *Stappia* and *Rhizobium* include species isolated from crude oil and known for aromatic hydrocarbon degradation such as O-xylene and phenanthrene (Prince et al., 2010). The *Bacillus* genus, also observed in module 2, contained several species, i.e. *Bacillus cereus*, *Bacillus sphericus*, *Bacillus pumilis* and *Bacillus halodenitrificans*. Finally, some pathogenic bacteria, i.e. *Vibrio* and *Arcobacter* genus, were present in the module 4A (Zettler et al., 2013; Lehner et al., 2005).

The plastic surfaces before and after enrichment were observed by SEM (supporting method 4) revealing holes and cracks on several LDPE film surfaces containing *Alcanivorax borkumensis* and *Microbulbifer* (Fig. 5). The most numerous signs of degradation were observed on the "PFS4" sample. On the polymer-type plastics (PS and PET), it was not possible to observe any degradation as the negative control surface was very irregular and already contained cracks.

3.4. LDPE degradation by Alcanivorax borkumensis

Alcanivorax borkumensis was isolated as described in supporting method section to test its capacity to form a biofilm and to degrade LDPE. First, its capacity to form biofilm on PET, LDPE, PS in hexadecane medium (0.75%) was assessed after 24 h, 96 h and 144 h of incubation at 30 °C. Control samples correspond to LDPE films immersed in the medium without bacteria and undergoing exactly the same treatments as the samples. As shown in Fig. 6, after 24 h of growth, no difference between the different plastics was observed. Nevertheless, after 96 h and 144 h a significantly higher percentage of biofilm associated bacteria was observed on LDPE, 68.5% and 68.1% respectively, than the two other plastics, 17.7% and 34% for PET and 20.5% and 29.61% for PS (Fig. 6A). Interestingly, simultaneously to the biofilm formation, preliminary results showed a significantly plastic weight loss of 3.5% +/- 0.34 after 80 days showing the ability of its bacterium to degrade LDPE (Fig. 6B, C). Interestingly, similar loss of weight was also observed in medium containing 0.05% of yeast extract and LDPE as main carbon source after 80 days. Moreover, the apparition of oxidative peaks in ATR-FTIR spectra after 80 days confirms chemical modifications/ degradations of LDPE (Fig.S14).

4. Discussion

4.1. Structure of the marine plastisphere community

The nature of the macro-plastics sampled in Corse (Fig.S7 and Table S5 was in agreement with previous studies showing that the two most common plastics in oceans are PE and PE (Debroas et al., 2017; Rios

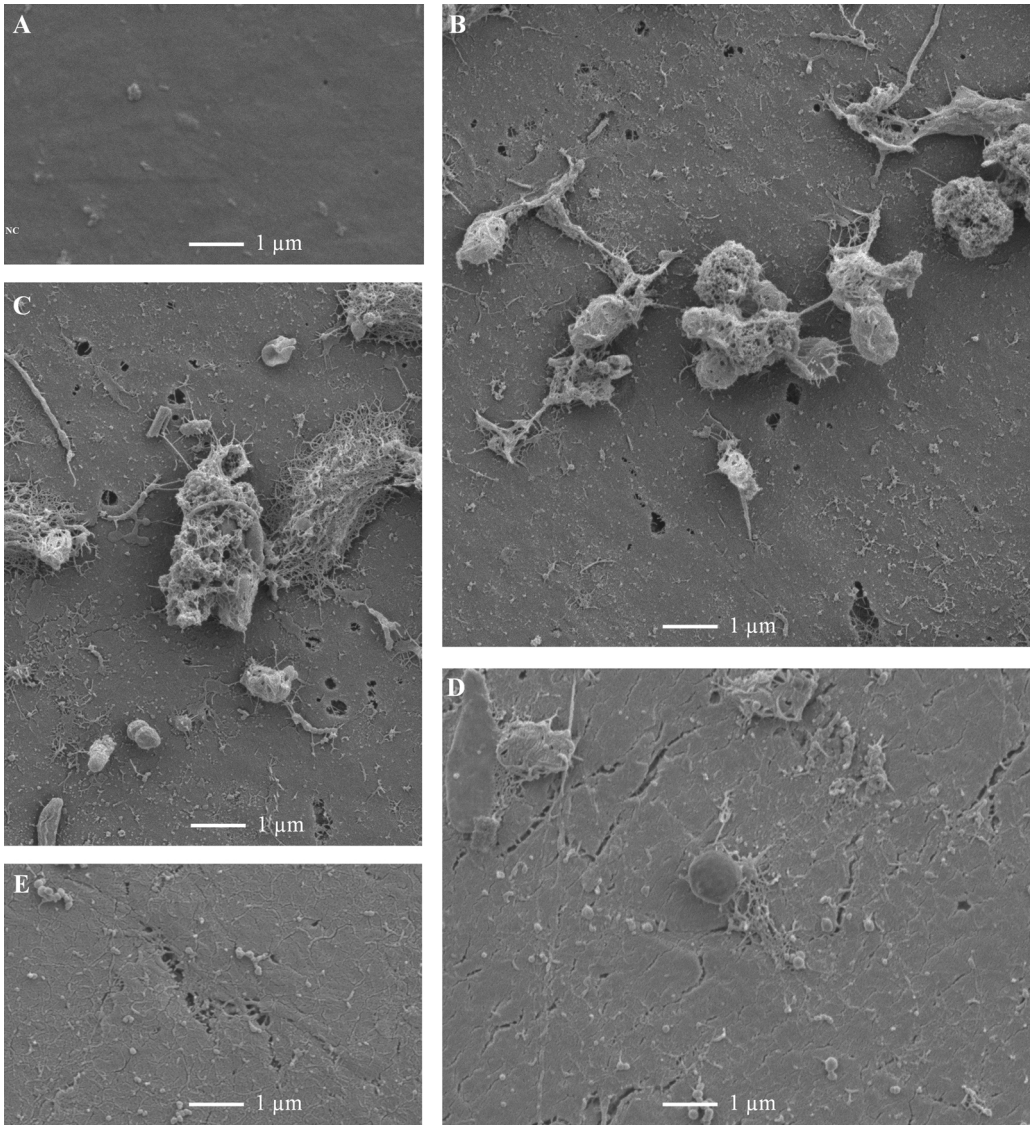

Fig. 5. Scanning electron microscopy images of the LDPE surface after two months of enrichment. (A) The negative control (NC) was immersed for 2 months in medium without bacteria. The other pictures show biofilm on LDPE: (B & C) PFS4 LDPE and (D & E) PH4 LDPE. Some degradation signs can be observed. The NC does not contain these deformations.

et al., 2007; Morét-Ferguson et al., 2010; Woodall et al., 2014). These plastics, mostly found on the surface, have specific gravities of approximately 0.94 (PE) and 0.84 (PP), which are lower than the specific gravity of sea water (approximately 1.025). PVC, having a specific gravity higher than that of sea water, is found on the sediment (1.38) (Andrady, 2011).

The lower richness and equitability of plastic associated biofilms showed the sharp selection of bacteria attaching to the plastic substrate in a comparison with those in the water column (Zettler et al., 2013) and sediments (De Tender et al., 2015). The decreased richness of plastic-associated communities can be explained by the higher amount of nutrient on plastic surface (Zettler et al., 2013). In a microenvironment containing a higher concentration of carbon, more selective and metabolically bacteria were expected on the plastic surface (Zettler et al., 2013). Regarding equitability, previous studies have shown a lower equitability in the surrounding environment than on plastics (Zettler et al., 2013), while our results highlight dominant bacterial species in plastic-associated microbiomes, which decrease the equitability in plastic-associated microbial communities.

Our results showed a distinctness between bacterial community from PFS and PH samples (Fig. 1A) but 17,92% of OTUs from sediment plastic were only shared with floating plastic. This observation confirms that plastics are a distinct environmental niche (Zettler et al., 2013) whose, despite the difference in plastic localization (surface *vs* sediments), host a specific "core" microbial community which was similar from one localization to another.

Other studies have shown the impact of localization and season on plastic-associated communities (Oberbeckmann et al., 2016, 2014; Jiang et al., 2018). For the first time, we showed differences between the microbial communities associated with plastics from the same geological region and from surface- and sediment-localized plastics. *Proteobacteria*, particularly *Gammaproteobacteria* and *Alphaproteobacteria* dominated plastic communities (Fig. 2). These groups are characteristic of the primary colonizers in a plastic biofilm in the marine environment, while *Bacteroidetes* are known to be secondary colonizers (De Tender et al., 2015). According to this information, the plastics sampled from the sediments were in the environment longer than the floating plastics. This is the only temporal information we can get from our plastic samples because we do not have any information about the immersion time of these samples, nor the growth time of biofilms. Moreover, the presence of *Verrucomicrobia* in plastic biofilm was relevant as previous studies have revealed *Verrucomicrobia* among

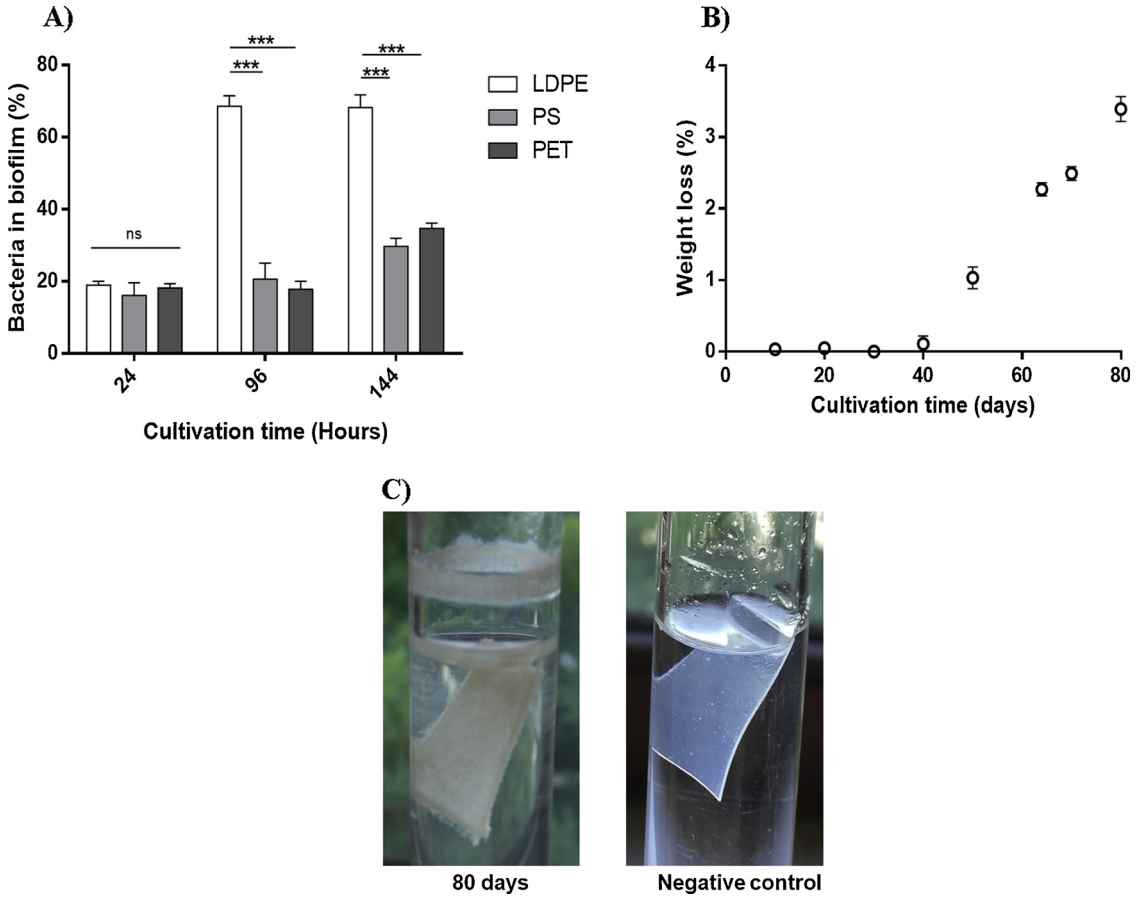

Fig. 6. (A) Percentage of bacteria in biofilm after 24H, 96H and 144 H of culture in 0.75% hexadecane medium quantified by crystal violet method; (B) Percentage of LDPE weight loss after 80 days of culture in medium containing 0.05% of hexadecane; (C) Biofilm pictures on LDPE after 80 days and the negative control in medium containing 0.05% of hexadecane. ANOVA with Tukey's post-hoc test; *, $p < 0.05$;**, $p < 0.01$;***, $p < 0.001$.

the most abundant phyla in plastic-associated biofilms in rivers (Hollein et al., 2014) and in marine environments (Zettler et al., 2013; Oberbeckmann et al., 2016). In the Mediterranean Sea, *Verrucomicrobia* is regularly present in sediment (Kühn et al., 2015). The presence of *Cyanobacteria* on PFS, that have previously shown their ability to take part to plastic-associated biofilms (Zettler et al., 2013; Debroas et al., 2017; Oberbeckmann et al., 2014), is probably correlated to their need in light for photosynthesis as light from sun is more accessible from the ocean surface than in deeper water. Finally, a different bacterial profile was observed on sample made in PE in comparison with 3 other replicates in PP with the abundant presence of *Epsilonproteobacteria* (Fig. 2, Table S5). Harrison and colleagues showed that low densities of PE microplastics are rapidly colonized in coastal sediment microcosms and that *Epsilonproteobacteria* dominate these communities (Harrison et al., 2014). Concerning SSH samples, *Gamma-*, *Alpha-* and *Deltaproteobacteria* were the most represented groups in sediments samples (Fig. 2), as previously observed in a bacterial community from Mediterranean sediments (Polymenakou et al., 2005).

The heatmap showed that *Vibrio* was specific to floating plastics (Fig.S4). The *Vibrionaceae* family, which comprised up to 20% of the floating plastic-associated bacterial community (Fig.S13), is often found in biofilms associated with plastic (Zettler et al., 2013; De Tender et al., 2015; Kirstein et al., 2016). *Vibrio* is known for extremely fast growth (Polz et al., 2006) explaining its ability to dominate the plastisphere, e.g., *Vibrio parahaemolyticus* has a doubling time of 15 min (Ulitzur, 1974). Nevertheless, *Vibrionaceae* is present on sedimentary plastic in low percentages (< 1%) in that study and can be completely absent in the coastal sediments (Bryant et al., 2016; Harrison et al., 2014). This genus contains animal and human pathogen strains. Therefore, Zettler et al. put forth that plastics serve as a vector of disease transmission by plastic ingestion by birds and fishes (Zettler et al., 2013; De Tender et al., 2015). Indeed, plastics can traverse significant distances across the ocean and transport invasive species, explaining why *Vibrionaceae* were mainly found on floating plastics, with few *Vibrionaceae* on sedimentary plastics. The lack of consistency in the representation of *Vibrio* on plastics could be explained by the fact that these microorganisms interact indirectly with plastics, acting as hitchhiker microorganisms that are not using plastics as carbon source (Debroas et al., 2017). Several OTUs were specific to sedimentary plastics such as *Pseudoalteromonas,* known for its association with diatoms on plastics (Oberbeckmann et al., 2016; De Tender et al., 2015). Some species from *Glaciecola* and *Winogradskyella* families specific to sedimentary plastics are known for their ability to degrade complex polysaccharides, such as xylan and cellulose (Klippel et al., 2011; Kang et al., 2013). An unclassified *Oceanospirillales* OTU was also specific from pH samples, order containing bacteria such as *Alcanivorax borkumensis*, are able to degrade hydrocarbons as the sole source of carbon (Yakimov et al., 1998). From this order, *Alcanivoraceae* was also discriminant in the microbial community on PET, as shown by Oberbeckmann et al. (Oberbeckmann et al., 2016).

4.2. Selection of potential candidates for plastic degradation

After 2 months of enrichment in presence of plastic as main carbon source, the decrease of equitability and richness can be explained by the "microcosm effect" and the strongly enrichment of specialist bacteria. Some signs of structural modifications of the surface were observed on LDPE films and not on the negative control which suggests that bacteria

should be able to degrade plastics. Nevertheless, further analysis will be necessary to confirm a degradation of these solid polymers.

Concerning the bacterial composition of the enrichment, as seen before, the most abundant family on several LDPE sample and specific from this polymer was *Alcanivoraceae*. This bacterium seemed to have a high affinity with LDPE. *Rhodobacteraceae* and *Flavobacteraceae* were also present. Interestingly, *Sulfitobacter* (included in R*hodobacteraceae)* is known to be abundant in PE biofilms after 35–44 weeks (De Tender et al., 2017a). Besides in the *Flavobacteriaceae* family, *Gramella* and *Arenibacter* were enriched on the LDPE-associated biofilms. These bacteria are able to degrade polymeric carbon sources (Bauer et al., 2006) and hydrocarbons (Gutierrez et al., 2016) and could therefore play a role in the degradation of LDPE. The *Marinobacter* genus, mostly present on one LDPE sample, contains some species, such as *Marinobacter hydrocarbonoclasticus*, that are able to degrade hydrocarbon (Gauthier et al., 1992). The genus *Vibrio,* already present on biofilm before the enrichment, seems to have a great affinity for different plastic types and can develop in the community in large amount despite low carbon resources. Consequently, *Vibrio* may be able to remain dormant on plastic, waiting for a new source of carbon. *Desulfovibrionaceae* was represented by only one genus, *Desulfovibrio*, involved in the biodegradation of xenobiotic compounds such as PE glycol (Pathak and Navneet, 2017). *Pseudomonas* genus is present in PS samples and several *Pseudomonas* species are known for their ability to degrade polystyrene (Mohan et al., 2016). Once again, *Marinomonas*, a hydrocarbon-degrading bacterium, was selected on PET, showing that species from this genus can degrade chrysene and phenanthrene, which are cyclic hydrocarbons (Melcher et al., 2002). Our results showed a strong selection of hydrocarbon degraders on plastics that suggests their ability to hydrolyze plastic, as previously suggest by Zettler et al. (2013).

The network analysis highlighted that *Vibrio* and *Arcobacter* are co-occurrent and present on the three plastics, suggesting that these OTUs are opportunistic bacteria with no role in polymer degradation. Results showed also the co-occurence of hydrocarbon degraders in the enrichment, e.g. *Winogradskyella*, *Gracilimonas* and *Zeaxanthinibacter* genera known to be overexpressed in the presence of hydrocarbons. In spite of that, until now, their implication in their degradation has never been shown (Newton et al., 2013). Several OTUs specific from PET (*e.g. Cellulosimicrobium, Stappia, Clostridiales* and *Rhizobium)* include species able to degrade aromatic hydrocarbon degradation such as O-xylene and phenanthrene (Prince et al., 2010). There seem that could have a link between the PET structure, containing aromatic cycle and the aromatic hydrocarbon degradation. Finally, the presence of Bacillus genus is relevant because some *Bacillus* species were involved in consortia able of PE degradation (Sudhakar et al., 2008) and in toluene degradation (Li et al., 2006). A large number of bacteria were simultaneously selected after two months of enrichment, including hydrocarbon degrading bacteria, some of which are specific to one type of plastic. These results highlight species association to be explored for their plastic degradation capabilities working with synthetic consortia. It would also be interesting to look at the functional aspect of this degradation process by studying in detail the metabolisms of the selected bacteria. In that clusters, *Alcanivorax borkumensis* seemed to be a good potential candidate for the plastic degradation with its strongly enrichment after 2 months on LDPE and it is also highlighted in the network analysis.

4.3. *Alcanivorax borkumensis, a potential LDPE-degrading bacterium*

The strongly enriched *Alcanivorax borkumensis* during the enrichment experiment using LDPE is a hydrocarbonoclastic bacteria, growing on alkanes (e.g., cyclo-alkanes, linear alkanes and isoprenoids). Different enzymatic systems, such as alkane hydroxylase (alkB1, alkB2), cytochrome P450, and ferredoxin, seem to be involved in hydrocarbon catabolism, permitting *A. borkumensis* to degrade a large range of alkanes from C5 to C32 (Golyshin et al., 2003; Sabirova et al., 2011). An alkane hydroxylase from *Pseudomonas* sp. (alk B), similar than alk B from *A. borkumensis*, is known to be involved in the degradation of low-molecular-weight PE. This bacterium also contains genes similar to those involved in halogenated alkane degradation in other bacteria and genes involved in the metabolic activation of polycyclic aromatic hydrocarbons (Sabirova et al., 2011; Noh et al., 2018). A recent study showed that polyesterases, enzymes from marine bacteria (including *A. borkumensis*), are capable of degrading biobased and synthetic polyesters (Hajighasemi et al., 2016). This bacterium seems to be an excellent candidate for the petroleum based plastic degradation such as LDPE. Moreover, *A. borkumensis* has a high affinity with the LDPE, the most hydrophobic plastic. By producing biosurfactants and modifying cell membrane hydrophobicity, *Alcanivorax* sp. is known to increase and modulate alkane bioavailability according to environmental conditions (Konieczna et al., 2018; Naether et al., 2013). Preliminary results of this present study showed that this bacterium is able to degrade LDPE with a degradation of 3.4% after 80 days. The efficacity of process is very limited but similar yield have been observed with other microorganisms such as fungi or other bacteria (Muhonja1 et al., 2018). No degradation was observed during the first 40 days probably because of the primary consumption of hexadecane (0.05%) at the beginning of the experiment.

An optimization of degradation conditions (temperature, supplemental carbon source, molecular weight of LDPE, UV treatment…) must be investigated and will allow to open new perspectives to use this bacterium alone or in consortium to recycle LDPE, one of more abundant petroleum-based plastic.

5. Conclusion

The present study discloses a 16S rRNA profile in the plastisphere of the Mediterranean Sea similar to those found in the other sea region. In addition, we showed that bacterial communities developing on floating and sedimentary plastics within the same geographical point (i.e., surface *vs* sediments) host distinct bacterial communities and that plastisphere hosts microbial communities were environmental distinct niche. An enrichment method was used to detect potential specific microbial degraders. Moreover, the bacterial communities able to form a biofilm on surfaces of synthetic polymers is clearly plastic nature dependent. Several genera containing hydrocarbon-degrading bacteria species were enriched on several plastics and species association were observed on LDPE films including *Alcanivorax borkumensis*. Our results showed clearly that *Alcanivorax borkumensis* is able to form large biofilms on the LDPE surface and was able to degrade it. If all studies revealed its ability to biomineralize petroleum hydrocarbon, we showed for the first time its potential capacity to degrade a solid structure based to alkane such as petroleum-based plastic LDPE. The mechanism of a partial depolymerization of LDPE - a crucial step for its degradation by *Alcanivorax borkumensis* - needs to be further investigated.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.120899.

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