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Plastic Degradation by Extremophilic Microbial Communities Isolated from Bulgaria and Russia

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Abstract. This work reports the potential impact of extremophiles in resolving one of the biggest contemporary ecological problem - accumulation of huge amounts of plastic pollutants. Lye (C1) and water mud (C2) from Atanasovsko ezero, Bulgarian salterns were enriched for 2 weeks in a mineral salt medium provided with four synthetic plastics, Polycaprolactone (PCL), Polyvinyl alcohol (PVA), Polystyrene (PS), and Polypropylene (PP). Esterase activity was established for C1 on PVA and for C2 - on PCL and PVA. Scanning electron microscopy analysis of the plastics showed most explicit alterations on PCL surface. While the presence of C1 in the culture medium caused damage of the plastic surface, C2 interacted with it directly by forming a biofilm in surface breaks. Metagenomic analysis of C2, control and C2 with PCL revealed lower phylogenetic diversity in the presence of PCL and sharp rise of Gammaproteobacteria 16S rRNA sequences. The flourishing of the family *Halomonadaceae* was accompanied by a strong domination of the genus *Halomonas*, suggesting its active participation in PCL degradation. Thermophilic microorganisms in samples from Kamchatka hot springs were enriched in a medium with high- and low-density polyethylene in anaerobic conditions, at 78ºC. Degradation of polyethylene after incubation was monitored by SEM. Results of 16S rRNA genes profiling compared with control variants revealed a domination of bacteria of phylum *Dictyoglomi* and family *Thermoanaerobacteraceae* in anaerobic thermophilic Kamchatka enrichments in the presence of polyethylene. These results indicate the possibility of thermophilic anaerobes to degrade the two types of polyethylene.

Key words: synthetic plastics, plastic biodegradation, saltern environment, hot springs, halophilic community, thermophilic community, esterase activity, 16S rRNA, biofilm.

Abbreviations: Polycaprolactone - PCL, Polyvinyl alcohol - PVA, Polystyrene - PS, Polypropylene - PP, Scanning electron microscopy – SEM, transmission electron microscopy – TEM, high-density polyethylene – HDPE, low-density polyethylene - LDPE.

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Introduction

Plastics are man-made polymeric hydrocarbons characterized by high durability and low cost. The excellent features and advantages of these long-chain polymers contribute to economic growth, but their difficult degradation favors their accumulation in the natural environment as solid wastes remaining intact for a very long time, with some being broken down for hundreds of years (Mohanan et al., 2020). Anthropogenic wastes from fishing, tourism, and marine industries are the main reason for coastal and marine pollution (Veiga et al., 2016). The exponentially growing scientific interest in plastic accumulation and its impact on nature reflect the public awareness to one of the biggest problems of our society. For example, the amount of plastic polymers in the oceans has exceeded six-times compared to plankton, due to which aquatic birds and fishes are in danger (Auta et al., 2017). More than 80% of the total used plastics are synthetic, derived from petrochemicals, such as polyvinyl chloride, polyethylene, polypropylene (PP), polystyrene (PS), polyethylene terephthalate, polyurethane, and polycaprolactone (PCL) (Urbanek et al., 2018). Plastics belong to two main groups according to their degradation rate: non-biodegradable that are characterized by a slow rate process and biodegradable for which biodegradation is faster. The necessity of degradation of plastic wastes is obvious, however physical and chemical methods create various environmental hazards.

The growing amount of plastic waste creates selective pressure on microorganisms for the assimilation of the new substrates. Biodegradation is a process by which microbial organisms utilize plastics as a sole carbon source and decompose large polymer molecules to oligomers. It occurs after or in parallel with abiotic (physical and chemical) environmental factors influence as abiotic degradation weakens the strength and changes the structure of the polymers. However, as the evolutionary process was not accompanied so far by plastics presence in nature, the natural adaptation of microorganisms happens with a low degradation rate that could be accompanied by the risk of irreversible environmental changes (Debroas et al. 2017). It is important to consider the microbial degradation of synthetic polymers in order to understand what microorganisms are active in the biodegradation and the enzymes and mechanisms involved. A comparatively low number of reports tried to reveal the influence of plastic pollution on marine microbial life and the impact of microorganisms in the biodegradation process.

Extremophilic microorganisms develop a remarkable diversity of metabolism, however, little is known about their ability to break down synthetic polymer substrates (Atanasova et al., 2021a). Halophilic microorganisms are adapted to grow and thrive in saline environments, such as salterns, and high salt seawaters. Halophilic microorganisms grow at NaCl concentration of 1-3% (slight halophiles), 3-15% (moderate halophiles) or above 15% (extreme halophiles) (Ventosa et al., 2015). The most abundant moderate and extreme halophiles are members of two genera: *Halomonas* and *Chromohalobacter* (family *Halomonadaceae*), Gammaproteobacteria. Their natural niches are salterns, saline lakes, oceans, and coastal areas. The oceans are the largest saline environments with an average salinity of 3.5%, while hypersaline environments are derived from the evaporation of seawater and contain salt concentrations in excess of seawater reaching up to 35%. The information for plastic degrading marine communities is very scarce (Urbanek et al., 2018), although plastics become more and more persistent in the oceans. The reports on the composition of marine communities involved in plastic degradation have referred mainly to seawaters where salt concentration is not as high as the salterns one. These

investigations have showed many unique features and revealed flourishing community taxa in the presence of plastics suggesting their active role in the degradation process. The metagenome analysis could reveal the community structure and the role of different members in the biodegradation process as some of microorganisms have the capacity for degrading-enzymes production, while others could contribute to biofilm formation posing the substrate and enzyme producer in a close vicinity. The results from the metagenome analysis could also contribute metagenome screening for desired enzyme activities from the preferred microbial group.

Few reports of depolymerization of plastics by thermophilic bacteria are known, mainly related to the ability of thermophilic actinomycetes (*Thermobifida fusca, Thermomonospora curvata, Thermomyces insolens*) to degrade PET (Wei et al., 2014). The thermophilic bacterium *Brevibacillus borstelensis* reduced the molecular weight of PE by 30% in 30 days at 50 ° C (Hadad et al., 2005). Consortium of *Brevibacillus* sp. and *Aneurinibacillus* sp. degraded PE and PP at 50°C (Skariyachan et al., 2018). All the above mentioned microorganisms are aerobes; the ability of thermophilic anaerobes to degrade plastics so far has not been reported.

The aim of the current work is to investigate the potential of halophilic microbial communities isolated from Pomorie salterns, Bulgaria and thermophilic communities isolated from Kamchatka hot springs, Russia for plastic degradation.

Materials and Methods

Sample collection and culture enrichment

Two samples, lye (C1) and water mud (C2) containing plastic debris were collected from Atanasovsko ezero (33% salinity), a part of Burgas salterns (42°32'09"N 27°28'49"E), Burgas Bay, Black Sea, Bulgaria, and used for the inoculation of a mineral medium containing one of the four synthetic plastics – two biodegradable, polycaprolactone (PCL) and polyvinyl alcohol (PVA) and two non-degradable or very difficult to degrade – polystyrene (PS) and polypropylene (PP). The samples were collected in sterile glass bottles and stored at 4 °C during their transfer to the laboratory. Two grams lye or mud were used as an inoculum for enrichment in 20 mL basal medium containing (%): NH_4NO_3, 0.01; KH_2PO_4, 0.03; K_2HPO_4, 0.14; $MgSO_4$, 0.01; $FeSO_4.7H_20$, 0.002; $Na_2MoO_4.2H_2O$, 0.0005; NaCl, 15, yeast extract, 0.03, pH 7.3. The plastics were sterilized separately by three hours soaking in 96% ethanol and five minutes sonication at room temperature. They were added to the basal medium at a final concentration of 0.3%±0.02. The cultivation was performed at 30°C, 80 rpm for two weeks, then the culture medium was replaced with a fresh one and cultivation continued for another two weeks. The control flasks did not contain any plastic. Growth (OD_{660}) and esterase activity in the supernatant were monitored daily. Three flasks were used for each variant.

Samples of water and sediments from two Uzon Caldera (Kamchatka) hot springs with temperature 78-80^{o}C were used for the enrichment of anaerobic thermophilic prokaryotes able to degrade low density polyethylene (LDPE) and high density polyethylene (HDPE). The samples were collected in 2018 during the Extremophiles Metabolism Laboratory (Federal Research Center of Biotechnology RAS) expedition to Kamchatka. One spring was located at Eastern thermal field of Uzon Caldera, the temperature at the sampling site was 80°C and pH was 6.5-7.0. Another spring was located at the Central thermal field of Uzon Caldera, the temperature at the sampling site was 78-80°C and pH was 6.1. For the enrichment of anaerobic thermophilic prokaryotes 1 ml of each sample from two Kamchatkan hot springs was used for the inoculation with 8 ml of anaerobically prepared medium. The gas phase of the enrichments was filled with N_2. The medium composition was as follows (%): NH_4Cl, 0.1;

$MgCl_2.2H_2O$, 0,033; $CaCl_2.6H_2O$, 0.01; KCl, 0.033; KH_2PO_4, 0.05; resazurin, 0.0001; trace elements solution, 0.1; vitamin solution, 0.1 (Wolin et al., 1963); $NaHCO_3$, 0.05; Na_2S. $9H_2O$, 0.05, pH 7.0. The medium was prepared under N_2 and 8 ml of medium were poured into 15 ml vials supplemented with 100 mg of shredded LDPE or HDPE were. The headspace of the vials was filled with N_2, and after that the vials were hermetically stoppered. Inoculated tubes with and without polymeric substrates, as well as those with the same non-inoculated medium and with polymers were incubated at 78°C for 45 days.

Electron Microscopy

Plastic surface appearance after four weeks of cultivation with C1 and C2 was observed by SEM. Control samples of plastics in the absence of bacteria were processed in parallel. The samples were fixed for 2 h in 4% glutaraldehyde in 0.1M Na cacodylate buffer (pH 7.2), then washed and post-fixed in 1% OsO_4 for 1 h. Dehydration was performed in graded ethanol series. After sputter-coating with gold using Edwards sputter coater, the samples were examined by SEM (Philips scanning electron microscope), at accelerating voltage 30 kV.

In the experiments on extremely thermophilic degradation of polyethylene, the morphology of the LDPE surface after bacterial treatment was investigated by transmission electron microscopy (Philips TEM-301, Netherlands). Samples were prepared using the replica method. For better contrast, platinum (VUP-5, Russia) was thermally sprayed onto the sample surface in a vacuum at an angle of 30° and then carbon was sprayed on the surface. The Pt-C replica was removed from the sample using gelatin. Gelatin was dissolved in water, and the replica was caught on a microscopic grid. The analysis was carried out at an accelerating voltage of 60 kV.

Esterase assay

Esterase assay was used for the estimation of enzyme activity. It was measured in the supernatant after centrifugation of the culture liquid at 4,000 g for 15 min. Hydrolysis of p-nitrophenyl palmitate (p-NPP) as a substrate was determined spectrophotometrically as described previously (Gupta et al., 2002) at 30°C in 0.05 M sodium phosphate buffer, pH 7.0 at 405 nm. One unit of esterase activity was determined as the amount of enzyme needed to liberate 1 µM p-nitrophenol per one minute in the described conditions. The molar extinction coefficient for p-nitrophenol at pH 7.8 was found to be 3.39×10^3 /M.

Analysis of the Microbial Communities

The metagenomic analysis of C2 microbial community was determined after four weeks-cultivation in a basal medium without plastic (MKC-C) and in the presence of 0.3% PCL. Two hundred mL culture liquid was centrifuged and the pellet was sent to Eurofins Genomics Europe, Ebersberg, Germany for a metagenomic analysis. 16S targets were PCR amplified from sample DNA extracts using target specific NGS primers and analysed by Amplicon sequencing on the Illumina MiSeq platform. During bioinformatical analysis the sequences were sorted into sequence sets according to their similarity. Each set was represented by a master sequence. Comparison of each master sequence with entries in the nucleotide collection of the US National Center for Biotechnology Information, NCBI finally provided the taxonomical assignment and hence the species presented in the sample. If the taxonomical assignment could not be resolved on the species level, a higher taxonomic rank was reported. Taxons with a fraction of at least 0.1% of all assigned reads were reported.

High-throughput sequencing of the variable V4-region of 16S rRNA gene was applied for characterization of microbial communities in extremely thermophilic enrichments on LDPE and HDPE. DNA isolation from the samples was performed using DNeasy PowerLyzer Microbial Kit

(Cat. 12255-50, Qiagen, Germany) according to the manufacturer's instructions and included the step of bead beating on FastPrep-24™ 5G grinder and lysis system (MP Biomedicals, USA).

Amplicon libraries were prepared according to the scheme described in Gohl et al. (2016). To obtain V4 amplicons PCR was performed on a StepOne Plus Real-Time instrument (Thermo Fisher Scientific, USA) using qPCRmix-HS SYBR mixture (Evrogen, Russia) and the following primer system: forward primer 515F(5′-CAAGCAGAAGACGGCATACGAGATGT GACTGGAGTTCAGACGTGTGCTCTTCCG ATCT XXXXXXXXXXXX ZZZZ GTGBCAGCMGCCGCGGTAA-3′), consisting, respectively, of "5 ′ Illumina Linker Sequence", "Index 1", "Heterogeneity Spacer" (Fadrosh et al., 2014) and reverse primer Pro-mod-805R (5′-AATGATACGGCGACCACCGAGATCTAC ACTCTTTCCCTACACGACGCTCTTCCGA TCT XXXXXXXXXXXX ZZZZ GACTACNVGGGTMTCTAATCC-3′), consisting of "3 'Illumina Linker Sequence", "Index 2", "Heterogeneity Spacer" and Pro-mod-805R primer sequence (Merkel et al., 2019). Amplicons were purified using the Cleanup Standard kit (Cat. BC022, Evrogen, Moscow, Russia). The quality of the final libraries was assessed using electrophoresis in agarose gel. High-throughput sequencing of the libraries was performed with MiSeq Reagent Micro Kit v2 (300-cycles) MS-103-1002 (Illumina, USA) on a MiSeq sequencer (Illumina, USA) according to the manufacturer's instructions. Primary data analysis, preparation of the OTU table, and analysis of taxonomic composition were performed using the SILVAngs online data analysis service and Silva138.1 SSU database.

Results

Plastic degradation by halophiles

Samples C1 and C2 were used for inoculation of the medium with PCL, PVA, PS, or PP as a main carbon source. The comparison of the microbial growth with and without plastic revealed a higher optical density of the PP, PS and PCL media inoculated with C1, while C2 did not show better growth on any of the used plastics (Table 1).

The highest level of esterase activity was established in C1-PVA, C2-PCL and C2-PVA media. SEM analysis confirmed the higher resistance to the non-biodegradable PP and PS on the action of bacterial communities, unlike the activity against the biodegradable plastics (Fig. 1). Its data were in a good agreement with the results of esterase assay (Table 1).

The four-week cultivation in the presence of C1 of PP, PS and PCL resulted in the occurrence of different degree of alterations of the plastic surfaces. Squamous-like deformations occurred focally at the surface of PS. In the case of PP, small surface elevations occurred which locally evolved in bigger fluffy formations. The visual changes of the PCL comprised the release of surface-attached thin threads as well as the formation of fluffy elevations alike the ones observed on PP, but with bigger size. With all three plastics, no evidence of biofilm formation was obtained in the presence of C1.

Table 1. Growth and esterase activity of microbial communities from Burgas salterns.

Synthetic Plastics	C1		C2	
	Growth (OD660nm)	Esterase activity (U/ml)	Growth (OD660nm)	Esterase activity (U/ml)
Control	0.30	0	0.34	0
Polypropylene (PP)	0.51	0	0.10	0
Polystyrene (PS)	0.49	0	0.37	0
Polycaprolactone (PCL)	0.51	5	0.09	46
Polyvinyl alcohol (PVA)	0.14	33	0.11	50

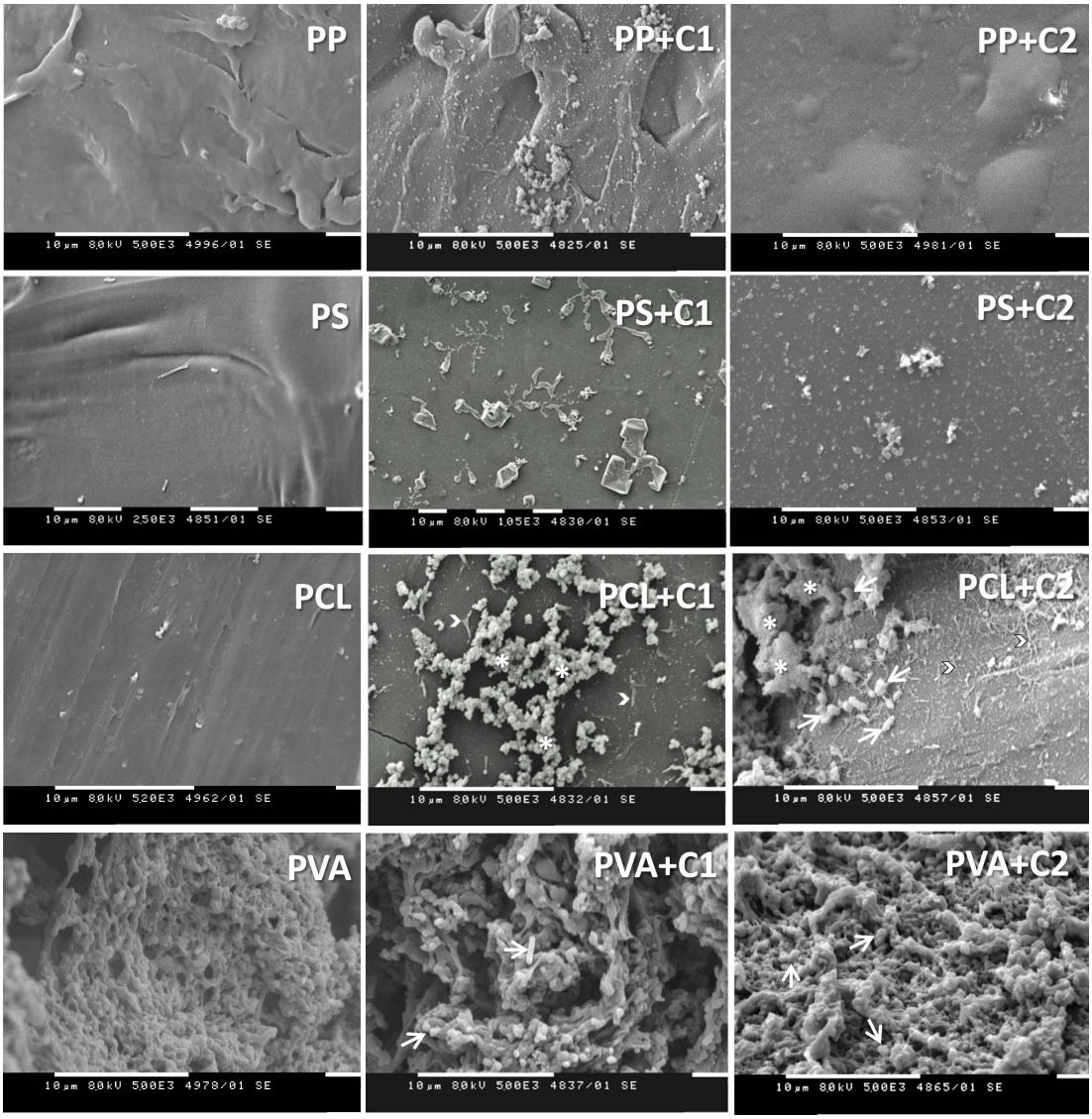

Fig. 1. Changes in the surface relief of the four examined types of plastics after four weeks incubation in the absence or presence of the bacterial communities C1 and C2. Arrows - bacterial cells. Asterisks, fluffy debris, deposited at the plastic surface. Arrowheads point to thread-like material on the plastic surface.

A comparison with the data in Table 1 implies that this community might interact with PP, PS and PCL by secreting in the culture liquid of enzymes (other than esterase) and utilizing for their growth the released degradation products. This community visually appeared to produce serious erosion of the surface of PVA where bacteria were seen to penetrate and attach to the plastic infolds. Together with the different pattern of changes in growth and esterase activity than the ones with the other three plastics, the data indicate a specific mode of interaction of C1 with PVA.

The presence of C2 in the culture medium did not apparently affect PP and caused some small deformations on PS. However, with PCL, surface-attached thread-like formations as well as cracks were formed at the plastic surface. The loci with threads and cracks served as foci of bacterial attachment, and bacteria adherent at the

surface and deeper in the cracks were visualized. Often, the openings of the cracks were overlaid with fluffy debris. The interaction of C2 with PVA was alike that described for C1. Altogether, the observation of bacterial attachment on PCL and PVA, on the background of the little growth in the liquid medium, and the increased esterase activity (Table 1) evidences the role of C2-biofilms in the interaction of the community with the two plastics.

Table 2. Taxonomic groups, identified in C2-C.

Identified group	Reads, %	
	C2-C	C2-PCL
Halomonadaceae	24.1	49.7
Hyphomonadaceae	19.5	3.8
Halomonas sp.	15.7	40.2
Alcanivorax sp.	13.0	0.2
Phyllobacteriaceae	4.9	0.8
Martelella sp.	3.1	1.0
Chromohalobacter sp.	2.9	2.4
Hyphomicrobiaceae	2.3	-
Pelagibacterium sp.	2.3	-
Methylophaga sp.	1.8	0.6
Marinicauda sp.	1.6	0.2
Algiphilus aromaticivorans	1.1	-
Proteobacteria	0.9	0.4
Rhizobiales	0.3	0.1
Rhodobacterales	0.1	-
Rhodobiaceae	0.1	-

The significant changes of PCL surface structure and the high esterase activity of C2-PCL determined our interest in the comparison its community structure with that of C2-Control by 16S rRNA profiling. In both communities all identified groups belonged to Alpha- and Gammaproteobacteria, however, the fraction of the two classes, Alpha- and Gammaproteobacetria differed significantly in both samples. Alphaproteobacteria dominated in C2-C (61.3%) while it represented only 6.9% in C2-PCL. At the same time significant domination of Gammaproteobacteria was observed for the last sample (93.1%). Seventeen phylogenetic groups were represented in the control (C2-C) while in C2-PCL they were twelve (Table 2). Under the selective pressure of PCL as a carbon source the share of the family *Halomonadaceae*, order *Oceanospirillales*, increased significantly – from 24.1% in C2-C to 89.9% suggesting the essential role of its representatives in PCL degradation process and a possible activity of the bacteria of genus *Halomonas*, as the number of its which 16S rRNA sequences increased almost three-fold.

Plastic degradation by thermophiles

The sampling sites were placed in two Uzon Caldera hot springs (Kamchatka). The light microscopy of the cultures supplemented with LDPE and HDPE showed the growth of two types of cells: very short rods and long thin rods after 45 days of incubation (Fig. 2). Transmission electron microscopy of LDPE surface from the enrichment culture and from non-inoculated control demonstrated a significant difference in the surface structure. LDPE from the control medium had a very visible thread structure, while after the incubation with thermal samples the surface of the polymer became smooth, probably due to microbial activity (Fig. 3).

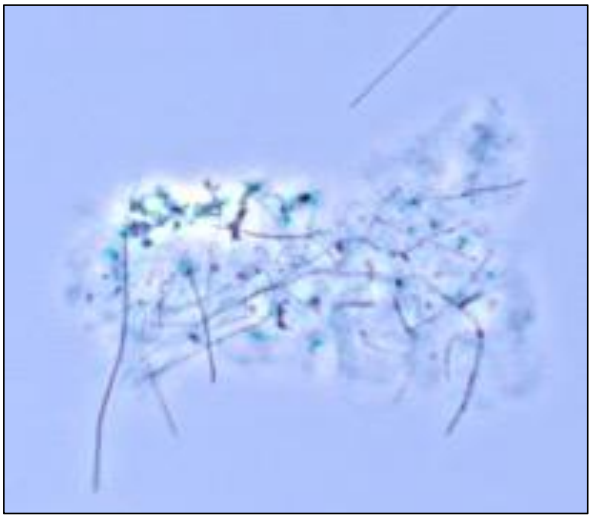

Fig. 2. Light microscopy of the enrichment culture obtained from Kamchatka hot spring with LDPE as a substrate.

High-throughput sequencing of 16S rRNA V3-V4 variable gene fragments showed that in the absence of the polymers microbial community contained representatives of genera *Thermodesulfobacter, Desulfovirgula, Fervidibacterium*, as well as members of phylum *Acetotermia* and uncultured Archaea (Fig. 4). Bacteria of genera *Dictyoglomus* (phylum *Dictioglomy*) and of genus *Caldanaerobacter* (phylum *Firmicutes*) represented 6 and 2%, respectively. In the enrichment culture supplemented with LDPE the bacteria of genera *Dictyoglomus* and *Caldanaerobacter* dominated, representing 64 and 23% of the whole community. The same picture was observed in the HDPE-supplemented enrichment (73 and 15%, respectively). These results are in agreement with the light microscopy of the enrichments where short rods (*Caldanaerobacter*) and long thin rods (*Dictyoglomus*) dominated.

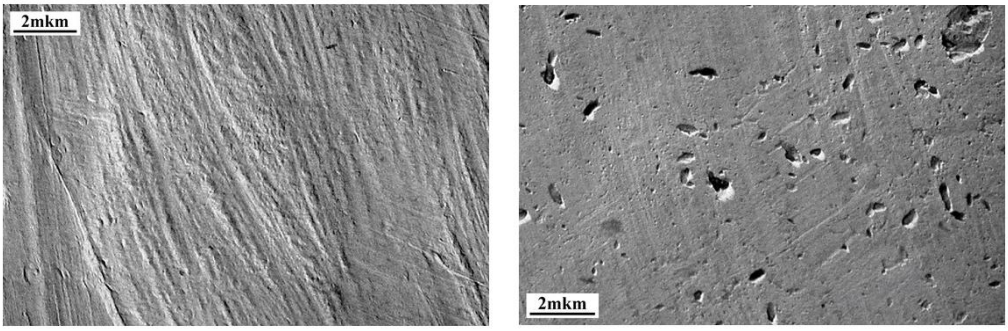

Fig. 3. TEM-images of LDPE surface after 45 days incubation at 78°C. A, Surface LDPE without inoculate; B, Surface of LDPE inoculated with thermal sample.

Discussion

Although the optical density of C1 in the presence of PP, PS and PCL was higher than in the control, no esterase activity was registered. These results could be explained by two reasons. Although some insignificant alteration of PP and PS surfaces was observed when C1 was cultured, these polymers are among difficultly degraded plastics (Pathak & Navneet, 2017; Urbanek et al., 2018) that probably required longer lasting experiments. The impossibility to register enzyme activity in our case could be due to a synthesis of very low enzyme amounts. Another possibility is that the active enzyme was not an esterase. Two groups of enzymes were identified as actively participating in PS and PP degradation, namely esterases and oxidoreductases (Mohanan et al., 2020). For example, P450 monooxygenase was involved in a saturated carbon-carbon bone cleavage reaction in the molecule of PS (Xu et al., 2019). An enzyme activity was registered in PVA culture liquid although the measured optical density was very low. This fact confirmed the opinion that the standard methods for the estimation of a bacterial growth, like the measurement of optical density or light microscopy cell counting are appropriate only in the case when the cells do not form biofilms on the plastic surface (Atanasova et al., 2021b). High levels of esterase activity in culture liquids of C2-PVA and C2-PCL were in a good correlation with the surface changes observed by SEM. Low OD of C2-PCL gives an evidence for a good biofilm formation ability of this community on PCL confirmed by SEM analysis. A good bacterial biofilm was observed after a fast colonization of plastic materials in seawater (Urbanek et al., 2018). The close vicinity between the substrate and microbial cells accelerates metabolic reactions that lead to the change

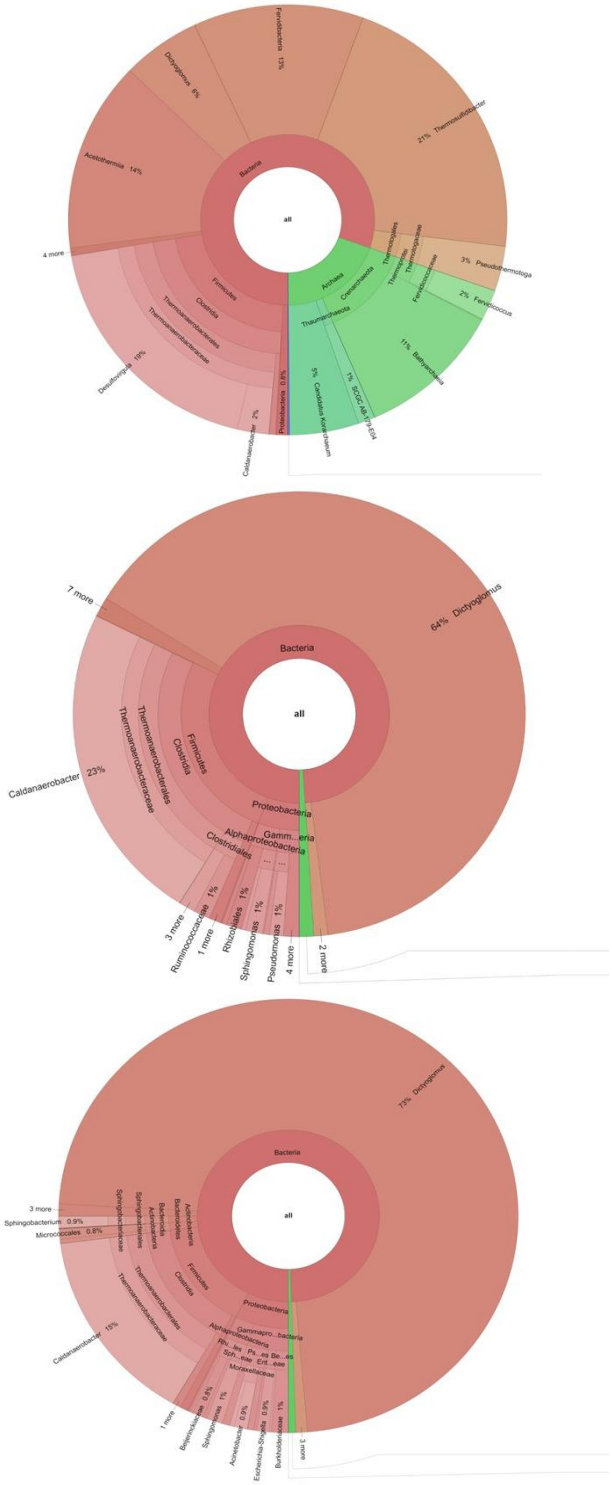

Fig. 4. Microbial diversity characterized by a high throughput sequencing of variable 16S rRNA fragments: a – initial sample from Uzon Caldera hot spring; b – microbial community after 3 weeks incubation with LDPE; c – microbial community after 3 weeks incubation with HDPE.

of plastic molecules followed by the breakdown of plastic itself (Harrison et al., 2011). Together with the nature of biofilm-forming microorganisms, the attachment at the surface and formation of biofilms depends on the surface structure and properties, like roughness, surface free energy, surface electrostatic interactions, and surface hydrophobicity (Rummel et al., 2017). Additional influence on biofilm development could be provoked by environmental conditions such as salinity, temperature, oxygen level, and limitation of light (Dash et al., 2013).

Analysis of the composition of C1 and C2 revealed the presence of 16S rRNA genes typical for halophilic environments. Higher density of bacteria attached to plastic wastes in comparison with free-living bacteria and bacteria attached to other organic particles in the natural saline environments, such as salt marshes and salt-rich industrial wastewaters was revealed by several researches. Similarly to the data in our investigations, a universal presence of representatives of the classes Gammaproteobacteria and Alphaproteobacteria, however in different proportions according to salinity, was observed in industrial water samples (Tourova et al., 2020a). A different community composition in biofilms formed on PS debris and industrial water was observed in Black Sea water at 10°C (Tourova et al., 2020b). These authors observed the family *Hyphomonadaceae* (mainly *Hyphomonas*) and the family *Erythrobacteraceae* (mainly *Erythrobacter*) as the most abundant across several stations in the coastal Baltic Sea. Dussud et al. (2018) reported the enhanced activity, higher numbers and bigger diversity of bacteria living on plastics in comparison with those living on organic particles and surrounding seawaters. Dominant microorganisms attached to plastic debris in Western Mediterranean Sea revealed a domination of *Cyanobacteria* (40.8%, mainly *Pleurocapsa* sp.) and Alphaproteobacteria (32.2%, mainly

Roseobacter sp. and *Erythrobacter* sp.). The most probable reason for the lower phylogenetic diversity observed in C2-PCL in comparison with other described marine communities and the different family and species domination (*Halomonadaceae*, 89.9%; mainly *Halomonas*, 40.2%) was the high salt concentration that restricted the number of the presented taxa. The observed dominant genus in our work differed from the genera commonly described as plastic degraders, like *Arthrobacter, Corynebacterium, Micrococcus, Pseudomonas, Rhodococcus*, and *Streptomyces* (Jackuin et al., 2019).

Most of the known microorganisms capable of polyethylene degradation are mesophilic aerobic organisms, including fungi and bacteria isolated from plastic contaminated soils, landfills and wastewater, as well as from sea water (Kotova et al., 2021). To date, only three thermophilic strains are known to decompose polyethylene at 50-60°C: *Aneurinibacillus* sp., *Brevibacillus* sp. (Skariyachan et al., 2018) and *Brevibacillus borstelensis* 707 (Hadad et al., 2005). In this work we found that anaerobic thermophilic bacteria of the genera *Dictyoglomus* and *Caldanaerobacter* are dominating in enrichment cultures with LDPE and HDPE. Representatives of both these genera are capable to hydrolyse complex organic substrates (Brumm et al., 2016; Kozina et al., 2010), however, they were never tested for the ability to degrade plastics. Bacteria of the genus *Dictyoglomus* are extreme thermophiles with the growth optimum in the temperature range from 70 to 75°C, and represent a deep phylogenetic lineage *Dictyoglomi* with only two cultured representatives. The genus *Caldanarobacter* is represented by moderate thermophiles of phylum *Firmicutes*. Further investigations are needed to prove that these two organisms are capable of using PE as the growth substrates; if confirmed it would be the first evidence on anaerobic PE degradation by thermophilic bacteria.

Conclusions

This work represents a first effort to elucidate the ability of saltern communities to degrade some synthetic plastics. Two communities isolated from Bulgarian salterns caused significant changes in the appearance of PVA after cultivation of C1 and C2, and PCL after cultivation on C2. The measured esterase activity was in a good agreement with the observed plastic surface changes. The analysis of the C2 microbial community composition with and without plastic revealed the selective pressure of the carbon source and the possible important role of the family *Halomonadaceae* representatives, and especially the species *Halomonas* in the process of PCL degradation. This result differed from other reported dominant taxa in halophilic niches. As *Halomonas* is among the most abundant genera in the seawater and salterns much hope is oriented to its active role in the development of future biodegradation processes in high salt environments.

The observed changes in polymer structure and microbial communities composition allow us to make an assumption on the ability of anaerobic organotrophic extremely thermophilic bacteria to degrade the polyethylene of low and high density.

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References

Atanasova, N., Stoitsova, S., Paunova-Krasteva, T. & Kambourova, M. (2021a). Plastic degradation by extremophilic bacteria. *International Journal of Molecular Sciences*, *22*, 5610, doi: 10.3390/ijms22115610.

Atanasova, N., Paunova-Krasteva, T., Stoitsova, S., Radchenkova, N.,

Boyadzhieva, I., Petrov, K., & Kambourova, M. (2021b). Degradation of poly(ε-caprolactone) by a thermophilic community and *Brevibacillus thermoruber* strain 7 Isolated from Bulgarian hot spring. *Biomolecules, 11*, 1488. doi: 10.3390/biom11101488.

Auta, H. S., Emenike, C. U. & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environment International, 102*, 165–176. doi: 10.1016/j.envint.2017.02.013.

Brumm, P. J., Gowda, K., Robb, F.T. & Mead, D.A. (2016). The complete genome sequence of hyperthermophilic *Dictyoglomus turgidum* DSM 6724TM reveals a specialized carbohydrate fermentor. *Frontiers in Microbiology, 7*, 1979. doi: 10.3389/fmicb.2016.01979.

Dash, H. R., Mangwani, N., Chakraborty, J., Kumari, S. & Das, S. (2013). Marine bacteria: potential candidates for enhanced bioremediation. *Applied Microbiology and Biotechnology, 97*, 561–571. doi: 10.1007/s00253-012-4584-0.

Debroas, D., Mone, A. & Ter Halle, A. (2017). Plastics in the North Atlantic garbage patch: a boat-microbe for hitchhikers and plastic degraders. *Science of the Total Environment, 599-600*, 1222–1232. doi: 10.1016/j.scitotenv.2017.05.059.

Dussud, C., Meistertzheim, A., Conan, P., Pujo-Pay, M., George, M., Fabre, P., Coudane, J., Higgs, P., Elineau, A., Pedrotti, M., Gorsky, G. & Ghiglione, G. F. (2018). Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. *Environmental Pollution, 236*, 807–816. doi: 10.1016/j.envpol.2017.12.027.

Fadrosh, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M. & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome, 2*, 6. doi: 10.1186/2049-2618-2-6.

Gohl, D., MacLean, A., Hauge, A., Becker, A., Walek, D. & Beckman, K. (2016). *An optimized protocol for high-throughput amplicon-based microbiome profiling*. Protocol Exchange, licenze. doi: 10.1038/protex.2016.030.

Gupta, N., Rathi, P. & Gupta, R. (2002). Simplified para-nitrophenyl palmitate assay for lipases and esterases. *Analytical Biochemistry, 311*, 98–99. doi: 10.1016/s0003-2697(02)00379-2.

Hadad, D., Geresh, S., & Sivan, A. (2005). Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *Journal of Applied Microbiology, 98*, 1093-1100. doi: 10.1111/j.1365-2672.2005.02553.x.

Harrison, P., Sapp, M., Schratzberger, M. & Osborn A. M. (2011). Interactions between microorganisms and marine microplastics: a call for research. *Marine Technology Society Journal, 45*(2), 12–20. doi: 10.4031/MTSJ.45.2.2.

Jacquin, J., Cheng, J., Odobel, C., Pandin, C., Conan, P., Pujo-Pay, M., Barbe, V., Meistertzheim, A. L. & Ghiglione, J. F. (2019). Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the "Plastisphere". *Frontiers in Microbiology, 10*, Article 865. doi: 10.3389/fmicb.2019.00865.

Kotova, I. B., Taktarova, Yu. V., Tsavkelova, E. A., Egorova, M. A., Bubnov, I. A., Malakhova, D. V., Shirinkina, L. I., Sokolova T. G. & Bonch-Osmolovskaya, E. A. (2021). Microbial degradation of plastics and approaches to make it more efficient. *Mikrobiologiya, 90*, №6 (in press).

Kozina, I. V., Kublanov, I. V., Kolganova, T. V., Chernyh, N. A. & Bonch-Osmolovskaya, E. A. (2010). Caldanaerobacter uzonensis, an anaerobic thermophilic heterotrophic

bacterium isolated from a hot spring. International *Journal of Systematic and Evolutionary Microbiology, 60*, 1372-1375. doi: 10.1099/ijs.0.012328-0.

Merkel, A. Y., Tarnovetskii, I. Y., Podosokorskaya, O. A. & Toshchakov S. V. (2019). Analysis of 16S rRNA primer systems for profiling of thermophilic microbial communities. *Microbiology, 88*, 671–680. doi: 10.1134/S0026261719060110.

Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. Frontiers in *Microbiology, 11*, 2837. doi: 10.3389/fmicb.2020.580709.

Pathak, V. M. & Navneet, S. (2017). Review on the current status of polymer degradation: A microbial approach. *Bioresources and Bioprocessing, 4*, 15, doi: 10.1186/s40643-017-0145-9.

Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D. & Schmitt-Jansen, M. (2017). Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science & Technology Letters, 4*, 258–267. doi: 10.1021/acs.estlett.7b00164.

Skariyachan, S., Patil, A. A., Shankar, A., Manjunath, M., Bachappanavar, N. & Kiran, S. (2018). Enhanced polymer degradation of polyethylene and polypropylene by novel thermophilic consortia of *Brevibacillus sp.* and *Aneurinibacillus sp.* screened from waste management landfills and sewage treatment plants. *Polymer Degradation and Stability, 149*, 52-68. doi: 10.1016/j.polymdegradstab.2018.01.018.

Tourova, T. P., Sokolova, D. S., Nazina, T. N., Gruzdev, D. S. & Laptev, A. B. (2020a). Phylogenetic diversity of microbial communities from the surface of polyethylene terephthalate materials exposed to different water environments. *Microbiology, 89*, 96–106. doi: 10.1134/s0026261720010154.

Tourova, T., Sokolova, D., Nazina, T., Grouzdev, D., Kurshev, E. & Laptev, A. (2020b). Biodiversity of microorganisms colonizing the surface of polystyrene samples exposed to different aqueous environments. *Sustainability, 12*, 3624. doi: 10.3390/su12093624.

Urbanek, A. K., Rymowicz, W. & Mirończuk, A. M. (2018). Degradation of plastics and plastic-degrading bacteria in cold marine habitats. *Applied Microbiology and Biotechnology, 102*, 7669-7678. doi: 10.1007/s00253-018-9195-y.

Veiga, J. M., Fleet, D., Kinsey, S., Nilsson, P., Vlachogianni, T., Werner, S., Galgani, F., Thompson, R. C., Dagevos, J., Gago, J., Sobral, P.& Cronin, R. (2016). *Identifying sources of marine litter.* MSFD GES TG Marine Litter Thematic Report; JRC Technical Report; EUR 28309. doi: 10.2788/018068.

Ventosa, A., de la Haba, R. R., Sánchez-Porro, C. & Papke, R. T. (2015). Microbial diversity of hypersaline environments: A metagenomic approach. Current Opinion in *Microbiology, 25*, 80–87. doi: 10.1016/j.mib.2015.05.002.

Wei, R., Oeser, T. & Zimmermann, W. (2014). Synthetic polyester-hydrolyzing enzymes from thermophilic actinomycetes. *Advances in Applied Microbiology, 89*, 267-305. doi: 10.1016/B978-0-12-800259-9.00007-X.

Wolin, E. A., Wolin, M. J. & Wolfe R. S. (1963). Formation of methane by bacterial extracts. *Journal of Biological Chemistry, 238*, 2882–2888. doi: 10.1016/S0021-9258(18)67912-8.

Xu, J., Cui, Z., Nie, K., Cao, H., Jiang, M., Xu H., Tan, T. & Liu, L. (2019). A quantum mechanism study of the C-C bond cleavage to predict the bio-catalytic polyethylene degradation. Frontiers in *Microbiology, 10*, 489. doi: 10.3389/fmicb.2019.00489.

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