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(Article begins on next page)

1 **Microbiome enrichment from contaminated marine sediments unveils novel**
2 **bacterial strains for petroleum hydrocarbon and heavy metal bioremediation**

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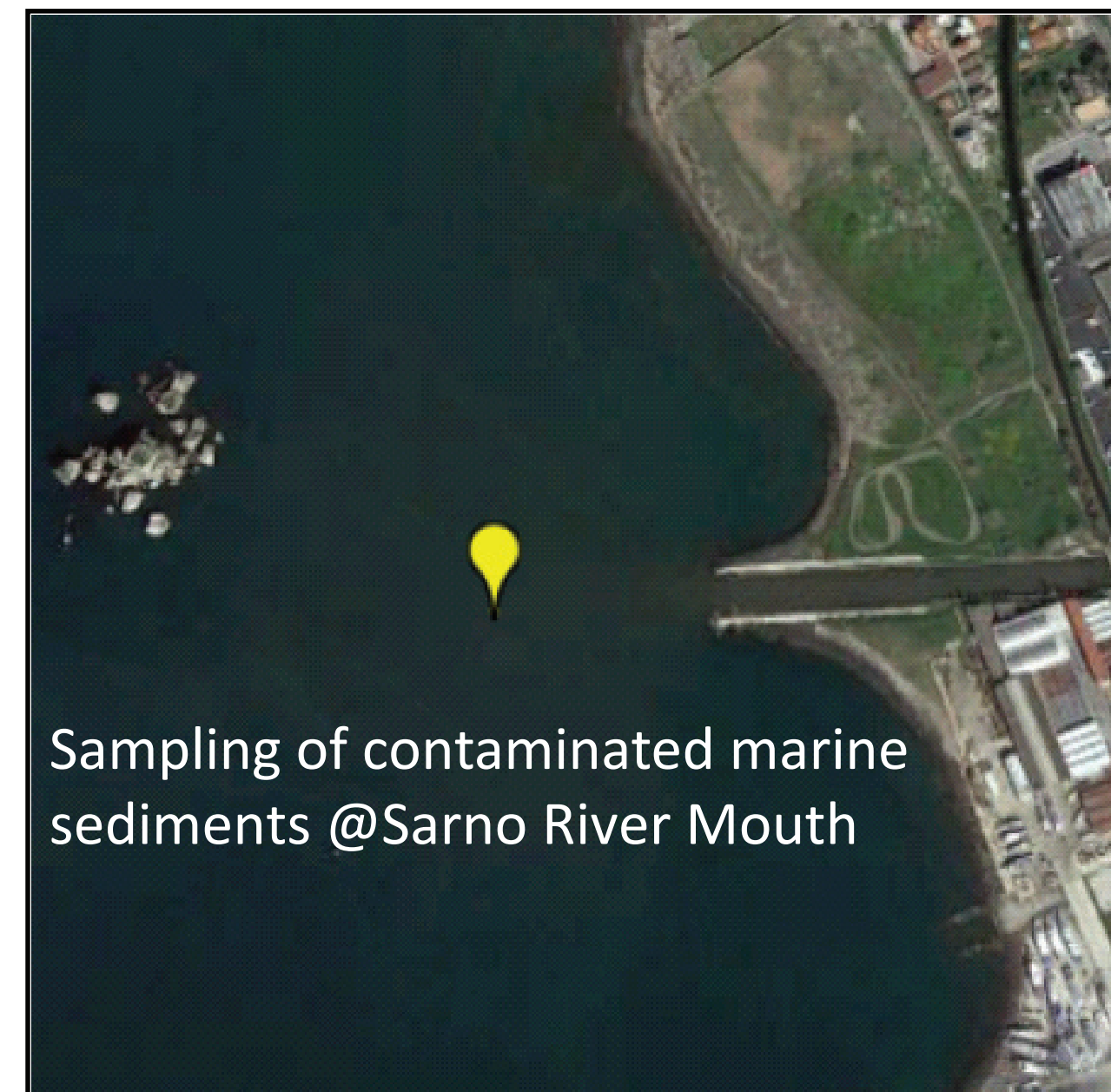
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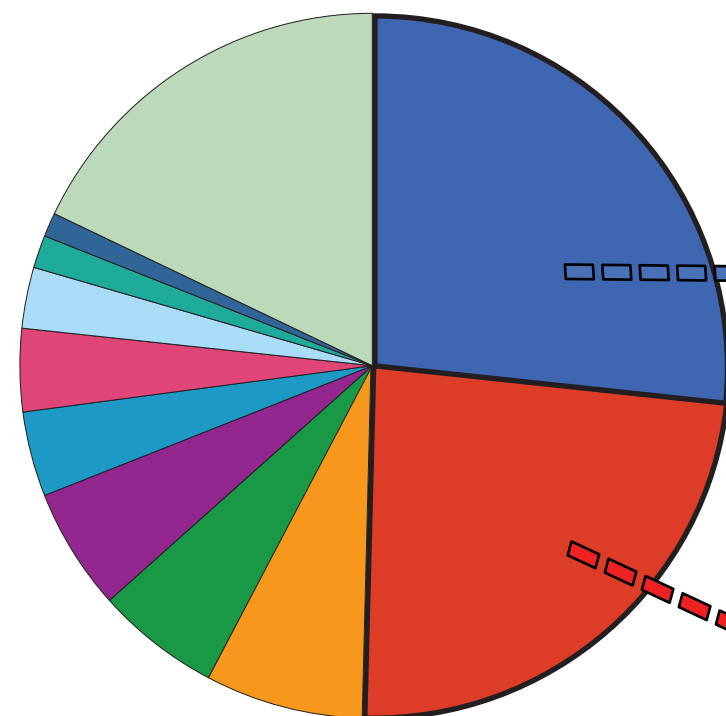
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Microbiome enrichment

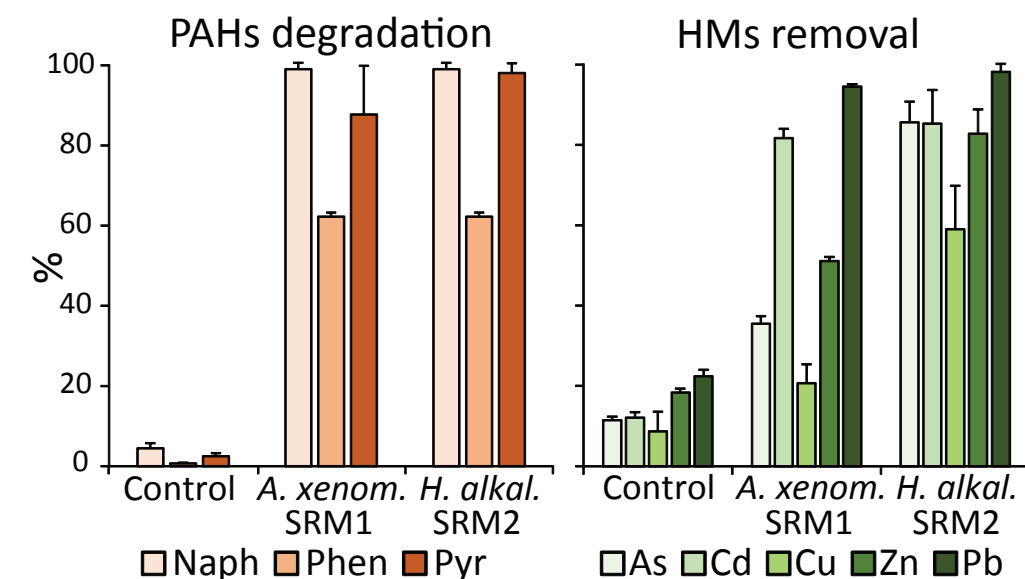


- **Halomonas (26.7%)**
- **Alcanivorax (23.7%)**
- Chromohalobacter (7.3%)
- Alkaliphilus (5.7%)
- Marinobacter (5.6%)
- Pseudomonas (3.9%)
- Ruegeria (3.8%)
- Pseudoalteromonas (2.8%)
- Burkholderia (1.5%)
- Marinomonas (1.1%)
- Others (<1%)

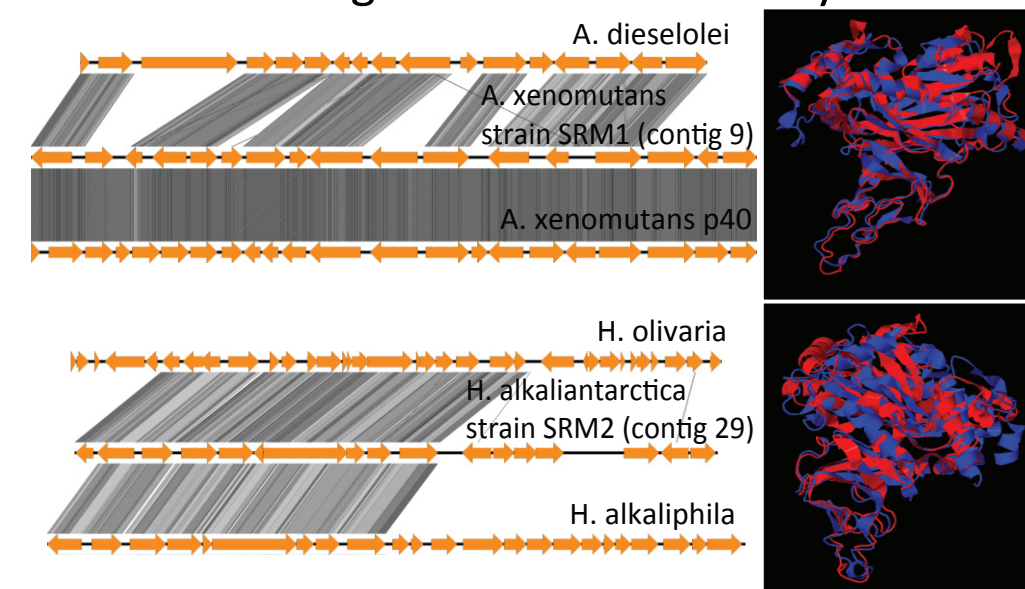
Bacterial isolation



Bioremediation experiments



Genomic basis of bioremediation and screening for novel useful enzymes



22 **Abstract**

23 Petroleum hydrocarbons and heavy metals are some of the most widespread contaminants affecting
24 marine ecosystems, urgently needing effective and sustainable remediation solutions. Microbial-
25 based bioremediation is gaining increasing interest as an effective, economically and environmentally
26 sustainable strategy. Here, we hypothesized that the heavily polluted coastal area facing the Sarno
27 River mouth, which discharges >3 tons of polycyclic aromatic hydrocarbons (PAHs) and ~15 tons of
28 heavy metals (HMs) into the sea annually, hosts unique microbiomes including marine bacteria useful
29 for PAHs and HMs bioremediation. We thus enriched the microbiome of marine sediments,
30 contextually selecting for HM-resistant bacteria. The enriched mixed bacterial culture was subjected
31 to whole-DNA sequencing, metagenome-assembled-genomes (MAGs) annotation, and further sub-
32 culturing to obtain the major bacterial species as pure strains. We obtained two novel isolates
33 corresponding to the two most abundant MAGs (*Alcanivorax xenomutans* strain-SRM1 and
34 *Halomonas alkaliantarctica* strain-SRM2), and tested their ability to degrade PAHs and remove
35 HMs. Both strains exhibited high PAHs degradation (60-100%) and HMs removal (21-100%) yield,
36 and we described in detail >60 genes in their MAGs to unveil the possible genetic basis for such
37 abilities. Most promising yields (~100%) were obtained towards naphthalene, pyrene and lead. We
38 propose these novel bacterial strains and related genetic repertoire to be further exploited for effective
39 bioremediation of marine environments contaminated with both PAHs and HMs.

40 **Keywords:** polycyclic aromatic hydrocarbons; heavy metals; bioremediation; next-generation
41 sequencing; marine biotechnology.

42 **1. Introduction**

43 Pollution of coastal environments due to organic (e.g., polycyclic aromatic hydrocarbons, PAHs)
44 and/or inorganic (e.g., heavy metals, HMs) contaminants can determine major detrimental effects on
45 the marine food web and human health (Tashla et al., 2018; Fuentes-Gandara et al., 2018; Loflen et
46 al., 2018; Buah-Kwofie et al., 2018). PAHs, HMs and metalloids are known to strongly affect
47 biological systems such as cell membranes, organelles and enzymes, causing cell cycle alteration,
48 carcinogenesis or apoptosis (Tchounwou et al., 2001; Sutton et al., 2002; Yedjou and Tchounwou,
49 2007a,b; Beyersmann and Hartwig, 2008; Patlolla et al., 2009; Kim et al., 2015; Costa et al., 2022).
50 Such contaminants can persist in the environment for a long time and can be subjected to
51 bioaccumulation and/or biomagnification processes, which increase their potential to cause harm
52 (Oyetibo et al., 2017). Conventional methods for the removal of PAHs and HMs include chemical
53 treatments (e.g., precipitation, oxidation and reduction), electrochemical techniques or physical
54 adsorption (Fenyvesi et al., 2019). Unfortunately, such methods typically produce special wastes
55 (e.g., toxic sludge and by-products) and are expensive, ineffective at low pollutant concentrations and
56 highly energy-demanding (Joshi, 2017; Priyadarshanee and Das, 2021). The use of microorganisms
57 (especially, bacteria, fungi and microalgae) may be a promising alternative or complementary
58 strategy to such conventional tools, due to several microbial characteristics including high
59 biodegradation/detoxification efficiency towards several contaminants, high surface area-to-volume
60 ratio and the ability to grow at high concentrations of toxic pollutants (Zouboulis et al., 2004;
61 Kordialik-Bogacka and Diowksz, 2014). Microbial-based remediation strategies are also considered
62 to be one of the most sustainable approaches due to low carbon footprint of the overall
63 decontamination process (Kuppusamy et al., 2017; Dell'Anno et al., 2020; Jain et al., 2022).
64 Microbes effectively degrade PAHs by several mechanisms including assimilation, intracellular
65 detoxification, and/or co-metabolism mediated by the activity of specific enzymes such as oxygenase,
66 dehydrogenase and ligninolytic enzymes (Johnsen et al., 2005; Ladino-Orjuela et al., 2016; Saravanan
67 et al., 2021). Microbial-mediated HMs' sorption, leaching or transformation are well-known as

68 inexpensive and highly efficient HM bioremediation processes (Leung et al., 2000; Aryal et al., 2010;
69 Sahmoune, 2018; Quiton et al., 2018; Cai et al., 2019). Co-contamination by PAHs and HMs is
70 typically encountered in most marine polluted sites (El-Mufleh et al., 2014; Ali et al., 2021) and
71 several microbes can exert a combined action towards such contaminants by bioleaching, biosorption
72 and biodegradation, which increase their overall removal and/or detoxification capacity (Liu et al.,
73 2017; Ali et al., 2021). Examples of this synergistic approach include microbial secretion - under
74 mixed PAHs and HMs contamination - of enzymes with useful bioremediation activities (Chen et al.,
75 2020) and of extracellular polysaccharides (EPSs). EPSs are particularly relevant targets for
76 bioremediation studies, as they consist of a wide array of polymers and functional groups, that can
77 simultaneously bind metals (thus enhancing HM extraction/removal from contaminated matrices)
78 (Amoozegar et al., 2012; Ates, 2015; Little et al., 2014; Mohite et al., 2017; Gupta and Diwan, 2017;
79 Cao et al., 2022; Cheng et al., 2022), and increase PAH solubilization and enzymatic degradation
80 efficiency (Gutierrez et al., 2013; Alaba et al., 2018).

81 Environmental matrices that are naturally or experimentally enriched with organic and/or inorganic
82 contaminants are an underexploited source of novel microbes resistant to contaminants and involved
83 in their biodegradation/detoxification (Beolchini et al., 2009; Adams et al., 2015; Fodelianakis et al.,
84 2015; Dell'Anno et al., 2021; Wang et al., 2021). Currently, high-throughput sequencing technologies
85 help to uncover such microbial bioremediation potential, providing novel insights on the diversity of
86 useful environmental microbes and their repertoire of genes involved in PAH and HM bioremediation
87 (Czaplicki and Gunsch, 2016; Dell'Anno et al., 2021; Meng et al., 2022; Hassan et al., 2022; Sharma
88 et al., 2022).

89 In this study, we selected the highly anthropically-impacted and severely contaminated coastal area
90 at the mouth of the Sarno River (Gulf of Naples, Mediterranean Sea), one the most polluted rivers in
91 Europe discharging large amounts of PAHs and HMs into the sea (Montuori and Triassi, 2012;
92 Montuori et al., 2013). Based on such high inputs of contaminants at this site, we postulated that here,
93 marine sediments could host microbiomes enriched in bacterial taxa that typically characterize marine

94 areas affected by oil-spills and/or industrial activities, and possibly useful for PAHs and HMs
95 bioremediation. We collected marine sediments from one of the most polluted sites in this area, and
96 we adopted a laboratory approach based on the enrichment of the sediment microbiome, subsequent
97 selection and culturing of bacteria resistant to HMs, and next-generation sequencing coupled with
98 laboratory experiments to unveil their potential for PAHs and HMs bioremediation.

99

100 **2. Materials and Methods**

101 **2.1. Sediment sampling, microbiome enrichment and selection of bacteria useful for PAH and** 102 **HM bioremediation**

103 Sampling was performed at the mouth of the Sarno River (Fig. 1). Surface sediments were collected
104 by a Van Veen grab, placed into sterile Whirl-Pak bags (Nasco), and then stored at 4°C in the dark
105 until processing. One gram of sediment was added to a 1000 ml flask containing 200 ml of Marine
106 Broth (Difco, Marine Broth 2216). Inoculated flasks were mixed and incubated at 28°C in the dark.
107 After 2 weeks of incubation, the enriched microbiome was plated by streaking onto marine agar
108 (Difco, Marine Agar) added with a mix of Pb^{2+} (500 ppm), As^{3+} (500 ppm), and Cd^{2+} (10 ppm) and
109 incubated at 28°C for 48 hours. Above HMs were selected as the most relevant in the study area, and
110 the applied concentrations were significantly higher than those determined in the sediments
111 (Montuori et al., 2013), to isolate HM-resistant bacteria with possible PAH and HM bioremediation
112 ability. PAHs were not added at this stage, as we hypothesized that bacterial PAH degraders were
113 already abundant in the original contaminated sediments, and our rationale was to select those able
114 to also tolerate high HM concentrations. As the diversity of potential PAH-degrading bacteria is
115 potentially high in contaminated sediments, we acknowledge that alternative enrichment strategies
116 (e.g., contextual addition of PAHs at this stage) may have led to different/additional bacterial isolates
117 than those obtained in our study.

118 Following incubation, high microbial growth was observed, which was confirmed to be a multi-
119 species bacterial culture by preliminary Sanger sequencing of the 16S rRNA genes, hence analyzed
120 by whole DNA shotgun sequencing, as described below.

121

122 **2.2. Whole DNA shotgun sequencing and analysis of bacterial metagenome-assembled genomes** 123 **(MAGs)**

124 The total genomic DNA (gDNA) obtained from the enriched mixed culture was extracted with the
125 DNeasy Blood & Tissue kit, according to the manufacturer's instructions. The DNA concentration
126 was determined using the Qubit™ dsDNA HS assay kit with a Qubit fluorometer (Thermo Fisher,
127 Waltan, US). Sequence library preparation of gDNA was performed using the Nextera DNA Flex kit
128 (Illumina, Hayward, USA) with 1 ng input DNA according to the manufacturer's instructions. The
129 resultant libraries were sequenced on an Illumina MiSeq instrument using a MiSeq Reagent kit V2
130 (500 cycles) with a 10% phiX v3 spike, generating 2×250 bp reads. Preliminary metagenome
131 processing and taxonomic and functional annotation were performed in MG-RAST under default
132 settings (Meyer et al., 2008). Read assembly was performed using CLC Genomics Workbench
133 version 11. Briefly, the raw reads were trimmed and demultiplexed, and contigs ≤ 500 bp were
134 removed from the final assembly. Binning of metagenomic contigs was performed using MyCC (Lin
135 and Liao, 2016) while completeness and contamination of MAGs and genome quality were
136 determined using CheckM with the lineage-specific workflow and default parameters (Parks et al.,
137 2015).

138

139 **2.3. Isolation and identification of single bacterial strains**

140 To obtain single colonies of pure bacterial strains, the enriched mixed culture was re-plated on marine
141 agar (Difco, Marine Agar) added with HMs as described above, through serial dilution. After several
142 re-streaking cycles of 48-hours incubation at 28°C, two main colony morphologies were observed,
143 and each re-streaked until confirmed to be pure by PCR analysis and Sanger sequencing targeting

144 16S rRNA gene (by universal bacterial primers E9F-5'-GAGTTTGATCCTGGCTCAG-3' and
145 U1510R-5'-GGTTACCTTGTTACGACTT-3'; Rodriguez-Caballero et al., 2012). All polymerase
146 chain reactions (PCR) were carried out in a Perkin Elmer Thermocycler (Gene Amp PCR system
147 6700) in a 50 µl reaction volume containing 1× PCR buffer, 200 µM of each dNTP, 0.5 µM of each
148 primer, 0.2 U of Taq Gold polymerase (Applied Biosystems, Waltham, MA, US) and 1 ng of template
149 DNA. Thermal cycling conditions were 5 min denaturation at 94 °C; 30 cycles of 94 °C for 30 s, 55
150 °C for 30 s and 72°C for 90 s; final elongation step at 72°C for 5 min. The PCR products were
151 analyzed by agarose gel (1.2% w/v) electrophoresis in TAE buffer solution (40 mM Tris-acetate, 1
152 mM ethylenediaminetetraacetic acid, EDTA) containing 0.5 µg ml⁻¹ (w/v) ethidium bromide. The
153 amplicons were purified and sequenced using an ABI PRISM 377 automated sequencer (Applied
154 Biosystems). The sequencing data were processed using Chromas Pro v. 1.5a software
155 (Technelysium, South Brisbane, QLD, Australia) for alignment and manual editing of sequences. The
156 consensus sequences of the isolates were compared with those deposited in GenBank using BLAST.
157 The two bacterial strains were matched with the two corresponding MAGs obtained by whole-DNA
158 shotgun sequencing by comparing their 16S rRNA gene sequences following nucleotide alignment
159 performed through a local blast in the annotation system. The pure bacterial strains were then tested
160 in experiments to assess their bioremediation ability to degrade PAHs and remove HMs, as described
161 below.

162

163 **2.4. Laboratory tests to assess the PAH degradation and HM removal ability of the bacterial** 164 **isolates**

165 Each bacterial isolate was incubated in flasks (T175, TPP tissue culture flasks, final volume 250 ml
166 Marine Broth; starting inoculum of 8 x 10⁷ cells ml⁻¹), and subjected to two treatments. These
167 included: i) addition of naphthalene, pyrene and phenanthrene (ratio of 1:1:1 with a total
168 concentration of 242 ppm); ii) addition of arsenic (As³⁺; 14 ppm), lead (Pb²⁺; 331 ppm), cadmium
169 (Cd²⁺; 1 ppm), copper (Cu²⁺; 74 ppm), and zinc (Zn²⁺; 899 ppm). Controls were included for each of

170 the two treatments, following the same procedure but without bacterial inoculum. All experimental
171 microcosms were set up in triplicate. The concentration of the toxic compounds used for
172 bioremediation experiments was selected based on the average values found in the surrounding
173 marine area (Montuori and Triassi, 2012; Montuori et al., 2013). The flasks were incubated for 27
174 days at 28°C, and bacterial growth was monitored by OD₆₀₀ at days 0, 9 and 27. The quantification
175 of PAHs and HMs was conducted, respectively, by gas chromatography-mass spectrometry (GC-MS;
176 EPA8270) (Casillo et al., 2018), and by inductively coupled plasma atomic emission spectroscopy
177 (ICP-OES; EPA6010) (EPA, 2014) on aliquots from each experimental treatment. The PAHs
178 degradation yield was calculated for each experimental treatment by comparing the concentrations of
179 each contaminant at the beginning and at the end of the incubations. For HMs, aliquots of each
180 experimental treatment at the end of incubations were first centrifuged (1000 x g, 5 min), and the HM
181 concentration in the pellet was used to calculate the % of HMs removal, as the % of HM mass in the
182 pellet compared to the HM mass added at the beginning of incubations.

183

184 **2.5. MAGs functional annotation and comparative genomics**

185 The genome taxonomy database (GTDB) (<https://gtdb.ecogenomic.org/>) implemented through K-
186 Base (www.kbase.us) was used to perform the whole-genome based classification of the two MAGs
187 obtained following whole-DNA shotgun sequencing and corresponding to the two bacterial strains
188 used in the PAHs degradation and HM removal experiments in this study. Species relatedness was
189 evaluated through the average nucleotide identity (ANI) analysis (Rodriguez and Konstantinidis,
190 2016; Han et al., 2016) by comparing the de novo genomes with the genomes selected following the
191 GTDB output. The obtained genomes were annotated by RAST (Overbeek et al., 2014) providing an
192 automated functional annotation or hypothetical protein annotation for each open reading frame
193 (ORF) identified on the genome. KEGG was used for metabolic prediction (Kanehisa et al., 2017).
194 Following automated identification of genes involved in hydrocarbon degradation or metal
195 resistance/detoxification/removal, manual verification of the annotated ORFs was conducted against

196 the SwissProt database. In addition, the sequences flanking the genes of interest were manually
197 annotated to better understand their genomic context, accurately delineate the regions involved in
198 these functions and for synteny comparison, and visualized using Easyfig (Sullivan et al., 2011). The
199 superimposition analysis of dioxygenases belonging to the two MAGs have been performed using
200 the web portal for protein modelling, prediction and analysis Phyre2 (Kelley et al., 2015), and enzyme
201 similarity was assessed according to Zhang and Skolnick, 2004.

202

203 **2.6. Statistical analyses**

204 To test for differences in the experimental results, Student T and Fisher-Snedecor tests were carried
205 out using PAST3 software (Hammer et al., 2001).

206

207 **3. Results and Discussion**

208 **3.1 Characterization of the enriched microbiome and of the bacterial isolates tested for PAHs** 209 **and HMs bioremediation**

210 The shotgun sequencing of the total DNA, extracted from the selectively enriched mixed bacterial
211 culture obtained in this study from the contaminated marine sediments of the Sarno River mouth,
212 resulted in >3.1 million high-quality reads (average sequence length 201 ± 59 bp; Supplementary Table
213 S1). Taxonomic annotation of this enriched metagenome showed a dominance of two
214 Oceanospirillales (Gammaproteobacteria) genera: *Alcanivorax* (~24% of total reads) and *Halomonas*
215 (~27% of total reads) (Fig. 2A). Several other bacterial taxa were detected in the metagenome, though
216 at a much lower relative abundance. These included other Gammaproteobacteria (*Chromohalobacter*,
217 *Marinobacter*, *Pseudomonas*, *Pseudoalteromonas* and *Marinomonas*), Clostridia (*Alkaliphilus*),
218 Alphaproteobacteria (*Ruegeria*), and Betaproteobacteria (*Burkholderia*) (Fig. 2A). All of these
219 bacterial taxa have previously been reported to typically increase rapidly in abundance during oil
220 spills, to degrade hydrocarbons and to tolerate/detoxify heavy metals (Gutierrez et al., 2013;
221 Dubinsky et al., 2013; Kumar et al., 2019; Dell'Anno et al., 2021; Huo et al., 2014; Liu et al., 2019;

222 Ramasamy et al., 2020; Ghosh et al., 2022). This suggests that our microbiome enrichment strategy
223 was successful in selectively boosting autochthonous bacterial taxa with promising potential for
224 petroleum hydrocarbon and HM remediation. This was also supported by the preliminary functional
225 annotation of the metagenome (Fig. 2B), which highlighted that the functions putatively related to
226 hydrocarbon degradation and to resistance/interaction with heavy metals represented an important
227 portion of the overall reads count (>6%). The major role of such functional features was further
228 suggested by the fact that their representation was quantitatively similar compared to fundamental
229 cell processes such as cell respiration, DNA metabolism or membrane transport (Fig. 2B). It should
230 be noted that our enrichment approach, resulting in the virtual absence of Archaea in the enriched
231 metagenome, might have overlooked possible syntrophic relationships among bacteria and archaea
232 in the original sediments, whose relevance in petroleum hydrocarbons degradation has been
233 highlighted by recent independent studies (Liu et al., 2018, 2021; Harindintwali et al., 2022).

234 The reads assembly and MAGs reconstruction and annotation resulted in two dominant MAGs with
235 genome completeness between 99-100%, which were classified as *Alcanivorax xenomutans* and
236 *Halomonas alkaliantarctica* based on GTDB-Tk whole-genome based classification, and supported
237 by 16S rRNA gene sequence identities (Supplementary Tables S2-S4). The ANI analysis indicated
238 *Alcanivorax xenomutans* strain KS-293 (Barbato et al., 2015; ANI score 99.10%) and *Halomonas*
239 *alkaliantarctica* strain CRSS (Poli et al., 2007; ANI score 97.13%) as their respective closest
240 relatives. Two additional partial MAGs (4-32% completeness) were recovered from the metagenome
241 and classified in the genera *Pseudoalteromonas* and *Alkalphilus*, but these were not analysed further.

242 The completeness and coverage of the four reconstructed MAGs reflected the relative contribution of
243 their reads to the overall sequence count (Fig. 2A), further suggesting that the obtained enriched
244 mixed bacterial culture was dominated by *A. xenomutans* and *H. alkaliantarctica*, with minor
245 contribution by other bacterial taxa.

246 The sequential and selective sub-culturing from the enriched mixed bacterial culture allowed us to
247 obtain the two strains that matched the two full-reconstructed MAGs based on 16S rRNA gene

248 sequence identity (100%). We hence refer to the two novel strains and related genomes obtained in
249 this study as *A. xenomutans* strain SRM1 and *H. alkaliantarctica* strain SRM2 (Supplementary Fig.
250 S1, S2).

251 The laboratory tests conducted to assess their ability for PAHs and HMs remediation showed high
252 PAHs degradation (ranging for both strains from 60% for phenanthrene to 100% for both naphthalene
253 and pyrene; Fig. 3A), as well as high HMs removal yields (34-91% for As, 79-94% for Cd, 21-70%
254 for Cu, 50-89% for Zn, and 94-100% for Pb), with highest values observed with *H. alkaliantarctica*
255 strain SRM2 for As, Cu, and Zn; Fig. 3B). Notably, we observed that the culturing of *A. xenomutans*
256 strain SRM1 and *H. alkaliantarctica* strain SRM2 with addition of PAHs resulted in growth rates
257 almost double to those of control conditions (Supplementary Fig. S3), which agrees with previous
258 independent evidence that these bacterial genera include taxa able to exploit hydrocarbons to produce
259 cell biomass (Mnif et al., 2009; Rahul et al., 2014). Both strains exhibited resistance to the high
260 concentrations of mixed HMs tested in our study (>1300 ppm, considering the sum of As, Cd, Cu,
261 Zn, and Pb) (Fig. 3B, Supplementary Fig. S3), indicating that these strains can be particularly useful
262 for PAH biodegradation of marine matrices that are simultaneously highly polluted with different
263 HMs. Indeed, the use of bacterial strains able to both degrade organic contaminants and tolerate toxic
264 inorganic compounds present in the target matrix can help to reduce failure risk in bioremediation of
265 environments that display high loads of mixed toxic contaminants (Thompson et al., 2005; Nwuche
266 and Ugoji, 2008; Alisi et al., 2009; Tyagi et al., 2011; Dueholm et al., 2015).

267 Overall, these results confirm several previous reports showing that *Halomonas* spp. and *Alcanivorax*
268 spp. include members that display high resistance towards toxic organic and inorganic contaminants
269 (Rahul et al., 2014; Fu et al., 2018, Catania et al., 2018; Dell'Anno et al., 2020) and can degrade
270 PAHs (Budiyanto et al., 2018; Kadri et al., 2018).

271 The observed higher degradation rates of naphthalene and pyrene than phenanthrene (Fig. 3A) may
272 be counterintuitive, as it is generally assumed that bacterial biodegradation of low-molecular-weight
273 PAHs (such as naphthalene and phenanthrene, with ≤ 3 aromatic rings) occurs faster than for high-

274 molecular-weight PAHs (with ≥ 4 aromatic rings, like pyrene). Despite previous investigations
275 support the expected pattern of a higher PAH recalcitrance with increasing molecular weight (Yu et
276 al., 2005; Haritash and Kaushik, 2009; Thavamani et al., 2012; Nzila et al., 2021), other studies have
277 reported the opposite trend, similar to that observed in the present study (Sohn et al., 2004;
278 Wongwongsee et al., 2013; Vaidya et al., 2017, 2018). This can be explained by different enzymatic
279 and metabolic pathways among microbes, by differences in the specific chemical-physical conditions
280 applied in the laboratory (which can differentially influence the degradability of different PAHs;
281 Leahy and Colwell, 1990; Bagby et al., 2017), as well as by possible interactions among different
282 PAHs, such as inhibition or enhancement of the biodegradation of specific high-molecular-weight
283 PAHs in the presence of specific low-molecular-weight PAHs (Guha et al., 1999; Vaidya et al., 2018).
284 As PAHs-contaminated sites typically display mixtures of high- and low-molecular-weight PAHs
285 (Bezza and Chirwa, 2017), we can conclude that our two bacterial strains, able to enhance
286 biodegradation rates of high-molecular-weight PAHs in the presence of low-molecular-weight PAHs,
287 can represent a significant advantage for environmental bioremediation applications.

288 Regarding the ability of the two tested strains to tolerate and remove HMs from contaminated
289 matrices, our results confirm previous findings obtained using other *Halomonas* and *Alcanivorax*
290 species. *Halomonas elongate*, *Halomonas halophila* and others displayed 50-94% removal yields
291 towards Pb, Cd and/or Cr (Amoozegar et al., 2012; Murugavelh and Mohanty, 2012; Asksonthong et
292 al., 2018; Abdel-Razik et al., 2020), while Pb removal ability has already been documented for some
293 *Alcanivorax* sp. (da Costa Waite et al., 2016; Dell'Anno et al., 2020; Ramasamy et al., 2020).
294 Notwithstanding, the current knowledge on the promising application of *Halomonas* sp. and
295 *Alcanivorax* sp. for the bioremediation of HMs is still in its infancy and should be investigated further,
296 also including tests for larger sets of HMs (Pennafirme et al., 2015; Verma and Kuila, 2019; Cecchi
297 et al., 2021; Dell'Anno et al., 2020).

298

299 **3.2. Genetic basis for PAHs biodegradation**

300 Understanding the genetic basis and metabolic processes involved in microbial removal of petroleum
301 hydrocarbons is fundamental to optimize bioremediation strategies, enabling tailored amendments to
302 favor specific bacterial metabolism, genetic bioengineering, or discovery of useful
303 enzymes/compounds (Schneiker et al., 2006; Dell'Anno et al., 2021; Sharma et al., 2022). Our results
304 from high throughput sequencing allowed us to identify the genes and metabolic pathways potentially
305 involved in the bioremediation ability of the tested *A. xenomutans* strain SRM1 and *H.*
306 *alkaliantarctica* strain SRM2. In particular, several genes involved in the metabolism of aromatic
307 compounds were identified through the automatic functional annotation of their MAGs
308 (Supplementary Fig. S4). Even though the two strains possessed a different number of enzymes
309 involved in these metabolic pathways (36 in *A. xenomutans* strain SRM1, 61 in *H. alkaliantarctica*
310 strain SRM2; Supplementary Fig. S4), they performed similarly in the laboratory tests for PAHs
311 degradation (Fig. 3A,B). We cannot exclude that additional genes for enzymes known to be involved
312 in petroleum hydrocarbon degradation and apparently missing in the two MAGs were not identified
313 through automatic annotation, due to divergence of DNA sequences from those available in current
314 public databases. To gain a more complete picture of the main pathways for hydrocarbon degradation
315 in the two novel strains, we manually checked the organization of the genomic regions that contain
316 the genes of major interest, including those for hydrocarbon degradation peripheral pathways (*cis*-
317 hydroxylation and *trans*-hydroxylation pathways) and central degradation routes (catechuate,
318 protocatechuate, homoprotocatechuate, homogentisate and phenylacetic pathways), as detailed
319 below.

320 *3.2.1 Peripheral pathways for hydrocarbon degradation*

321 The *cis*-hydroxylation pathway usually starts following the activity of ring hydroxylating
322 dioxygenase enzymes (RHDs) (Peng et al., 2019), which have large (α) and small (β) subunits
323 (Kauppi et al., 1998). The alpha subunit (RHD α) contains two conserved regions (the [Fe₂-S₂] Rieske
324 center and the mononuclear iron-containing catalytic domain), which promote the incorporation of
325 molecular oxygen into the aromatic ring forming a *cis*-dihydrodiol. We found RHDs in both *A.*

326 *xenomutans* strain SRM1 and *H. alkaliantarctica* strain SRM2 MAGs (Fig. 4A-D). The sequence of
327 *A. xenomutans* strain SRM1 showed ~100% identity with that of *A. xenomutans* P40 (Fu et al. 2018)
328 (Fig. 4A). Comparison with *A. dieselolei* B5 (Lai et al., 2012) highlighted a lower correlation but still
329 high overall similarity (homology of $\geq 70\%$) with 11 genes (including the RHD gene) (Fig. 4A).
330 Other proteins involved in hydrocarbon detoxification and degradation processes were found in *A.*
331 *xenomutans* strain SRM1 when analyzing the flanking region of RHD, including glutathione s-
332 transferase, linear amide C-N hydrolase, aldo-keto reductase and nitrite reductase (Lloyd-Jones and
333 Lau, 1997; Al-Turki, 2009; Cao et al., 2015; Imperato et al., 2019; Salam and Ishaq, 2019). The
334 comparison of the sequences flanking the RHD in *H. alkaliantarctica* strain SRM2 MAG, with those
335 of close relatives (Fig. 4B) highlighted that in all genomes the genes encoding the RHD and
336 ferredoxin (both directly involved in the degradation of hydrocarbons), as well as serin
337 hydroxymethyl transferase, sarcosine oxidase α,β,γ -subunits and formyltetrahydrofolate
338 deformylase, were all located in the same genomic region. Such genomic organization supports
339 previous evidence that genes associated with glycine and serine metabolism are involved in
340 hydrocarbon degradation (Yan and Wu, 2017). The 3D superimposition analysis revealed a high
341 similarity for both RHDs with naphthalene 1,2 dioxygenases (Fig. 4C,D), which are enzymes
342 involved in the first ring hydroxylation of multiple PAHs, including naphthalene, phenanthrene,
343 anthracene, dibenzothiophene and fluorene (Park and Crowley, 2006). Although it will be necessary
344 to confirm the specific function of the RHDs identified, the results of 3D modeling suggest a similar
345 ability in degrading PAHs (based on TM scores of 0.89 and 0.73 respectively for *A. xenomutans* strain
346 SRM1 and *H. alkaliantarctica* strain SRM2). Surprisingly, such high similarity between the two
347 enzymes' structures corresponded to only 30% identity in their secondary sequences. This further
348 suggests that the lack of matches of our MAGs DNA sequences for some of the other major genes
349 involved in hydrocarbon degradation may actually be due to evolutionary genetic divergence from
350 currently known bacterial genomes deposited in public databases.

351 An alternative to the *cis*-hydroxylation pathway is represented by the *trans*-hydroxylation pathway,
352 in which the cytochrome P450 system (CYP450) catalyzes a *trans*-dihydrodiols formation by the
353 epoxidation of the aromatic ring by epoxide hydrolase (Moody et al., 2005). For the *A. xenomutans*
354 strain SRM1 MAG, we found highly conserved homologs of the CYP450 within the 3 closest
355 relatives retrieved from NCBI (100% identity with *Alcanivorax xenomutans* sp 40, and >70% identity
356 with *Alcanivorax* sp N3-2A and *Alcanivorax dieselolei* B5) (Fig. 5A). Within the same genome region
357 containing CYP450, we also found two genes involved in the detoxification and biodegradation of
358 xenobiotics: glutathione-disulfide reductase (Moron et al., 1979) and a Rieske domain non-heme
359 oxygenase (Barry and Challis, 2013). For *H. alkaliantarctica* strain SRM2, the BLASTp analysis of
360 the CYP450 sequence found within its MAG showed no homologs within the 3 most similar reference
361 sequences retrieved from NCBI (of *H. axialiensis* Althf1, *H. olivaria* TYRC17 and *H. aestuari* Hb3),
362 despite the flanking region included other conserved genes (e.g., cytochrome C, nitrogen metabolism
363 and membrane transporters genes) (Fig. 5B). As the abovementioned three closest relatives were not
364 isolated from matrices heavily polluted by petroleum hydrocarbons and do not show genetic bases
365 for PAHs degradation (Tsurumaki et al., 2019; Nagata et al., 2019; Kim et al., 2018), we can expect
366 that this CYP450 system acquired by this novel *H. alkaliantarctica* strain SRM2 may have conferred
367 specific PAH-degradation abilities to this strain. As CYP450s are broadly distributed across the tree
368 of life and are considered the most versatile biocatalysts in nature because of the wide variety of
369 substrate structures they can react with (Nelson, 2018; Yeom et al., 2021; Haas et al., 2022), further
370 studies are needed to test the possible degradation/detoxification activity of the enzymes we identified
371 towards other contaminants besides petroleum hydrocarbons.

372 3.2.2. Central pathways for hydrocarbon degradation

373 The activity of RHD generates salicylate that enters the catechol pathway, a classic bacterial central
374 hydrocarbon degradation route mainly found in proteobacteria and actinobacteria (Nešvera et al.,
375 2015). In both MAGs, we identified the salicylate hydroxylase, which catalyzes the formation of
376 catechol acid (a substrate of the catechol 1-2 dioxygenase enzyme; Nešvera et al., 2015), as well as

377 the terminal enzymes (β -keto adipate succinyl-CoA transferase or β -keto adipyl thiolase), which
378 convert β -keto adipic acid in acetyl-CoA and succinyl-CoA (Peng et al., 2008). Additionally, we found
379 a hydroxyquinol 1,2 dioxygenase (BLASTp e-value 0, score 1461, id. 95.5%) suggested to promote
380 the formation of β -keto adipic acid (Ferraroni et al., 2005). These findings suggest that the catechol
381 pathway could contribute to the observed PAH degradation ability of both strains.

382 Following CYP450-mediated trans-hydroxylation, the degradation of hydrocarbons proceeds *via* the
383 protocatechuate metabolic pathway, which starts with an hydroxybenzoate hydroxylase that
384 transforms 4-hydroxybenzoate into 3,4 hydroxybenzoate (Fuchs et al., 2011). This enzyme has been
385 identified only in *H. alkaliantarctica* strain SRM2 MAG (Supplementary Fig. S5), which also
386 possessed a protocatechuate 3,4-dioxygenase, 3-carboxy-*cis,cis*-muconolactone cycloisomerase and
387 4-carboxymuconolactone decarboxylase. Again, we cannot exclude that genes with similar function
388 but low sequence-similarity may be present also in *A. xenomutans* strain SRM1 MAG. Conversely,
389 both MAGs possessed the terminal enzymes of the protocatechuate pathway (including β -keto adipate
390 enol-lactonase, 3-oxo adipate CoA transferase, β -keto adipyl-CoA thiolase) capable of catalyzing the
391 production of succinyl-CoA. The comparison of the *H. alkaliantarctica* strain SRM2 gene sequences
392 involved in this pathway with their homologs, generally showed high conservation (>70%)
393 (Supplementary Fig. S5). To the best of our knowledge, this is the first evidence for genes associated
394 with the protocatechuate degradation pathway organized as an operon in a *Halomonas* sp. (Corti
395 Monzón et al., 2018). Further analyses are needed to assess if this feature is peculiar to the strains
396 considered here, or common across *Halomonas* spp. and/or other bacterial taxa.

397 Several other genes of the homoprotocatechuate pathway (Méndez et al., 2011) were also identified
398 in both MAGs (Fig. 6A,B). Notably, the genome region containing the genes for the
399 homoprotocatechuate pathway in *A. xenomutans* strain SRM1 was highly conserved with that of close
400 *Alcanivorax* sp. relatives (Fig. 6A), while that of *H. alkaliantarctica* SRM2 showed no apparent
401 homologs in currently known *Halomonas* sp. genomes (Fig. 6B). We thus suggest that deeper
402 investigation of the homoprotocatechuate pathway of *H. alkaliantarctica* strain SRM2 through

403 mutation, heterologous expression or proteomics may unveil novel mechanisms for PAH
404 biodegradation.

405 Finally, *H. alkaliantarctica* strain SRM2 also displayed a complete set of genes for the phenylacetic
406 pathway within a specific genomic region, highly conserved with closest *Halomonas* sp. genomes
407 available for comparison (Supplementary Fig. S6), indicating that this strain possesses a particularly
408 wide array of genes for PAHs degradation. Additional enzymes involved in the homogentisate
409 pathway (Arias-Barrau et al., 2004; Guazzaroni et al., 2013), were detected in both MAGs, including
410 maleylacetoacetate isomerase, fumarylacetoacetate hydrolase, and 4-hydroxyphenilpyruvate
411 dioxygenase (data not shown).

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413 **3.3. Genetic basis for HMs removal/detoxification**

414 The blastp search against the SwissProt database identified in both MAGs the genetic basis for
415 exopolysaccharide (EPS) biosynthesis, which may not only facilitate PAH degradation by enhancing
416 the efficiency of all abovementioned PAH-degrading enzymes (Gutierrez et al., 2013; Alaba et al.,
417 2018), but also confer metal-binding properties contributing to explain the observed ability of these
418 strains to resist to and to sequester HMs (Amoozegar et al., 2012; Gupta and Diwan, 2017; Cao et al.,
419 2022; Cheng et al., 2022). Specifically, a UDP-glucose 4-epimerase (e-value 0, score 1709, 97%
420 identity), a UDP-glucose pyrophosphorylase (e-value 7.5×10^{-180} , score 1300, 90% identity), and a poly-
421 beta-1,6-N-acetyl-D-glucosamine synthase (e-value 0, score 2011, 81% identity) were identified in
422 *A. xenomutans* strain SRM1, with the latter two also present in *H. alkaliantarctica* strain SRM2
423 (respectively, e-value 2.6×10^{-165} , score 1206, identity 77.3%, and e-value 0, score 1977, identity 86.1%).

424 A deeper insight on the production of EPS by the tested strains would likely allow optimizing of their
425 use for simultaneous bioremediation of PAHs and HMs in different environmental conditions.

426 The annotation of the two MAGs by the automated RAST pipeline, followed by blastp check against
427 the SwissProt database, shed light on further mechanisms possibly involved in the ability of both
428 strains to resist to and to remove/detoxify HMs (Supplementary Fig. S7). In particular, several genes

429 coded for efflux pumps that selectively and non-selectively regulate the transport of multiple metals,
430 including *czcA*, *czcB*, *czcC*, *czcD* able to transport cobalt, zinc and cadmium, as well as *corC*, specific
431 for the transport of cobalt and magnesium. In addition, several genes were identified encoding for
432 proteins involved in the detoxification of copper, including a copper chaperone, copper homeostasis
433 protein CutE and CutF, copper resistance protein B, C, D, copper ATP ase, Cu-sensing two-
434 component system response regulator and Cu-responsive transcriptional regulator. Notably, the
435 automated RAST pipeline also identified multicopper and blue multicopper oxidases (Supplementary
436 Fig. S7), which may be synergistically involved both in Cu-detoxification/sequestration and act as
437 laccase-like multicopper oxidases for the degradation of PAHs and other organic contaminants
438 (Cooksey, 1994; Arregui et al., 2019; Ramasamy et al., 2020; Zhang et al., 2020).

439 Several genes coding for proteins known to confer resistance to and/or detoxify arsenic were found
440 in both MAGs (Supplementary Fig. S7), and our manual annotation of flanking gene sequences
441 highlighted operon-like structures (Figure 7A-B) similar to “arsenic islands” previously described
442 (Wu et al., 2018). In *A. xenomutans* strain SRM1, this MAG region (Figure 7A) included an arsenic
443 resistance protein ArsH, arsenic transporter ArsB, arsenate reductase ArsC and ArsR regulator (Rosen
444 and Liu, 2009; Chang et al., 2018). This genome region of *A. xenomutans* strain SRM1 showed on
445 average $\geq 70\%$ similarity with its closest *Alcanivorax* sp. relatives (Figure 7A), indicating high
446 conservation of this As operon and flanking regions across different *Alcanivorax* species. Similarly,
447 in *H. alkaliantarctica* strain SRM2, the region comprising the arsenic operon displayed high
448 conservation with the homologous sequences of close *Halomonas* sp. relatives (Figure 7B). Notably,
449 all the ORFs of the arsenic resistance operon in *H. alkaliantarctica* strain SRM2 MAG are contiguous,
450 whereas additional ORFs (of un-identified function) are present in the three reference genomes, that
451 separate ORF 7 from ORFs 8-9. As the three reference *Halomonas* genomes were obtained from
452 matrices not contaminated by As (Nagata et al., 2019; Williamson et al., 2016) we can argue that this
453 observed simplification of the arsenic operon may have enhanced the ability of *H. alkaliantarctica*
454 strain SRM2 to detoxify/remove As. The functional implications of the observed differences in the

455 structure of the arsenic resistance operon across different *Halomonas* species remains to be further
456 investigated, to understand which gene asset may perform better for different bioremediation
457 purposes.

458 A complete mercury-resistance operon (Boyd and Barkay, 2012) was also identified in *H.*
459 *alkaliantarctica* strain SRM2 (Fig. 7C), whose coding sequence was highly conserved within the
460 closest relatives retrieved from NCBI (*H. axialiensis* Althf1 and *H. sp.* ZM 3, which were isolated
461 from HM-rich hydrothermal vents in the Pacific Ocean and from a mineral waste repository,
462 respectively; Dziejewit et al., 2013; Tsurumaki et al., 2019). The structure of the identified mercury
463 operon showed typical features identified in other *Halomonas* species (Boyd and Barkay, 2012),
464 including two transcriptional regulators MerR, a mercuric transport protein MerT (able to transport
465 Hg(II) to the cytoplasm), a periplasmic Hg-binding protein MerP, a mercuric reductase MerA, and an
466 organomercurial lyase MerB. Based on the presence of this Mer operon, and especially of MerP, we
467 can expect that, even if not directly assessed in our bioremediation tests, *H. alkaliantarctica* strain
468 SRM2 may also perform Hg²⁺ biosorption (Huang et al., 2003).

469 Notably, several of the genes of the arsenic and mercury operons we identified have been documented
470 to cross-react with other toxic metals. For instance, ArsH has been reported to also detoxify and
471 enhance the precipitation of chromium by reducing Cr(VI) to Cr(III) (Xue et al., 2014), ArsB to also
472 detoxify the hazardous metalloid antimony (Meng et al., 2004), and MerP to be involved in the
473 biosorption of other HMs such as nickel, chromium, copper and zinc (Kao et al., 2008; Hsueh et al.,
474 2017). This suggests that similar cross-reactivity with multiple HMs may contribute to explain the
475 overall high removal efficiency towards the different HMs observed in our bioremediation
476 experiments, possibly extending our findings to additional HMs not directly investigated here.

477 Nevertheless, we acknowledge that other processes such as extracellular electron transfer and
478 electrocatalysis, not assessed in the present study, may be involved in HMs bioremediation (Liu et
479 al., 2018) and as such deserve further investigations.

480

481 **4. Conclusions**

482 In summary, our multidisciplinary study based on an integrated approach that combines experimental
483 microbiome enrichment, next-generation sequencing and selective culturing, allowed us to obtain two
484 novel *Alcanivorax* and *Halomonas* strains with promising bioremediation potential. Both strains were
485 shown to be resistant to, and to detoxify or remove multiple PAHs and HMs, and hence represent
486 promising candidates for developing bioremediation applications (e.g., bioaugmentation or *ex situ*
487 treatments) in environments contaminated by combinations of toxic pollutants. Finally, the contextual
488 analysis of their genomic repertoire highlights the presence of genes and/or operons that are proposed
489 as possible bioengineering targets, to further enhance the observed ability of these or other bacterial
490 strains to serve for environmental bioremediation purposes.

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507 **CRedit authorship contribution statement**

508 AD, ER, and FD conceived the study. FD, MP, ER, and CS conducted the field work. FD, ER, LJZ,
509 and MT conducted the laboratory analyses. FD, ER, LJZ, MT, AC, EB, PNG and AD contributed to
510 data elaboration. ER and FD wrote the draft of the manuscript. All authors critically revised the article
511 and contributed to its finalization.

512

513 **Declaration of competing interest**

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

516

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524

525 **Appendix A. Supplementary data**

526 Supplementary data to this article can be found online at XXX

527 **Data Availability Statement**

528 The sequencing data of the two bacterial strains described in this work have been deposited in NCBI
529 (accession number: PRJNA899357). The bacterial strains obtained in our study are maintained at the
530 Stazione Zoologica Anton Dohrn, National Institute for Marine Biology, Ecology and Biotechnology,
531 and are available upon request of collaboration.

532

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1005 **Figure Legends**

1006 **Figure 1.** Map of the study area with the position of the sampling site at Sarno River Mouth
1007 (Tyrrhenian Sea; 40°43'42.01"N, 14°28'0.45"E).

1008

1009 **Figure 2A-B.** Taxonomic and functional annotation of the enriched metagenome. A) taxonomic
1010 classification at the genus level based on M5NR database (standard cutoff: alignment length 15bp; e-
1011 value e^{-5} ; id. 60%). B) functional classification based on Subsystems ontology (level 1; standard
1012 cutoff: alignment length 15bp; e-value e^{-5} ; id. 60%), with functions putatively related to hydrocarbon
1013 degradation and to resistance/interaction with heavy metals contained in the subsystems “stress
1014 response” and “metabolism of aromatic compounds” subsystems.

1015

1016 **Figure 3A-B.** Removal of PAHs and HMs by the two novel bacterial strains isolated from the
1017 enriched metagenome. A) Degradation of PAHs (naphthalene, phenanthrene and pyren) under control
1018 (no bacteria) and treated conditions (i.e., addition of *A. xenomutans* strain SRM1 or *H.*
1019 *alkaliantarctica* strain SRM2). B) Removal of HMs (As, Cd, Cu, Zn, Pb) under control (no bacteria)
1020 and treated conditions (i.e., addition of *A. xenomutans* strain SRM1 or *H. alkaliantarctica* strain
1021 SRM2). Reported are average values and standard deviations.

1022

1023 **Figure 4A-D.** Comparative genomics of genetic regions for peripheral pathways for hydrocarbon
1024 degradation with ring hydroxylating dioxygenases enzymes (RHDs). A) Comparison of contig 9
1025 from *A. xenomutans* strain SRM1 MAG (containing the ORF 4 encoding for RHD) with three closest
1026 sequences from the NCBI database. In the right, the complete list of genes encoded within contig 9
1027 (ORFs 1-10) and those encoded by the 3 other reference genomes (ORFs 11-29). B) Comparison of
1028 contig 29 from *H. alkaliantarctica* strain SRM2 MAG (containing the ORF 2 encoding for RHD)
1029 with three closest sequences from the NCBI database. In the right, the complete list of genes encoded
1030 within contig 29 (ORFs 1-17) and those encoded by the 3 other reference genomes (ORFs 18-30). C)
1031 Superimposition of RHD of *H. alkaliantarctica* strain SRM2 (in red) with naphthalene 1,2-
1032 dioxygenase crystal structure from *Pseudomonas* sp. strain C18 (in blue)
1033 [www.rcsb.org/structure/2hmm]. D) Superimposition of RHD of *A. xenomutans* strain SRM1 (in red)
1034 with naphthalene 1,2-dioxygenase crystal structure from *Rhodococcus* sp. NCIMB 12038 (in blue)
1035 [www.rcsb.org/structure/2b1x].

1036

1037 **Figure 5A-B.** Comparative genomics of genetic regions for peripheral pathways for hydrocarbon
1038 degradation with cytochrome P450 (CYP450). A) Comparison of contig 76 from *A. xenomutans* strain
1039 SRM1 MAG (containing the ORF 12 encoding for CYP450) with three closest sequences from the
1040 NCBI database. In the right, the complete list of genes encoded within contig 76 (ORFs 1-20) and
1041 those encoded by the 3 other reference genomes (ORFs 21-29). B) Comparison of contig 15 from *H.*
1042 *alkaliantarctica* strain SRM2 MAG (containing the ORF 6 encoding for CYP450) with three closest
1043 sequences from the NCBI database. In the right, the complete list of genes encoded within contig 15
1044 (ORFs 1-14) and those encoded by the 3 other reference genomes (ORFs 15-27).

1045

1046 **Figure 6A-B.** Comparative genomics of genetic regions for central pathways for hydrocarbon
1047 degradation with genes for the homoprotocatechuate pathway. A) Comparison of contig 65 from *A.*
1048 *xenomutans* strain SRM1 MAG (containing the ORF 7-17 encoding for the genes of the
1049 homoprotocatechuate pathway) with three closest sequences from the NCBI database. In the right,
1050 the complete list of genes encoded within contig 65 (ORFs 1-20) and those encoded by the 3 other
1051 reference genomes (ORFs 21-38). B) Comparison of contig 29 from *H. alkaliantarctica* strain SRM2
1052 MAG (containing the ORF 4-14 encoding for the genes of the homoprotocatechuate pathway) with
1053 three closest sequences from the NCBI database. In the right, the complete list of genes encoded
1054 within contig 29 (ORFs 1-17) and those encoded by the 3 other reference genomes (ORFs 18-47).

1055

1056 **Figure 7A-C.** Comparative genomics of genetic regions for heavy metal removal/detoxification. A)
1057 Comparison of contig 20 from *A. xenomutans* strain SRM1 MAG (containing the ORFs 11-18
1058 encoding the arsenic resistance operon-like genomic region) with three closest sequences from the
1059 NCBI database. In the right, the complete list of genes encoded within contig 20 (ORFs 1-18) and
1060 those encoded by the 3 other reference genomes (ORFs 19-24). B) Comparison of contig 68 from *H.*
1061 *alkaliantarctica* strain SRM2 MAG (containing the ORFs 2-12 encoding the arsenic resistance
1062 operon-like genomic region) with three closest sequences from the NCBI database. In the right, the
1063 complete list of genes encoded within contig 68 (ORFs 1-15) and those encoded by the 3 other
1064 reference genomes (ORFs 16-29). C) Comparison of contig 30 from *H. alkaliantarctica* strain SRM2
1065 MAG (containing the ORFs 6-11 encoding the mercury-resistance operon) with three closest
1066 sequences from the NCBI database. In the right, the complete list of genes encoded within contig 30
1067 (ORFs 1-15) and those encoded by the 3 other reference genomes (ORFs 16-23).



