Phthalic acid esters degradation by a novel marine bacterial strain *Mycolicibacterium phocaicum* RL-HY01: Characterization, metabolic pathway and bioaugmentation

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Abstract: Phthalic acid esters (PAEs) are one of the most widely used plasticizers and the well-studied environmental pollutants with endocrine disrupting properties. Investigation about PAEs in terrestrial ecosystem has been extensively conducted while the fate of PAEs in marine environment remains underexplored. In this study, a novel di-(2-ethylhexyl) phthalate (DEHP) degrading marine bacterial strain, *Mycolicibacterium phocaicum* RL-HY01, was isolated and characterized from intertidal sediments. Strain RL-HY01 could utilize a range of PAE plasticizers as sole carbon source for growth. The effects of different environmental factors on the degradation of PAEs were evaluated and the results indicated that strain RL-HY01 could efficiently degrade PAEs under a wide range of pH (5.0 to 9.0), temperature (20 °C to 40 °C) and salinity (below 10%). Specifically, when Tween-80 was added as solubilizing agent, strain RL-HY01 could rapidly degrade DEHP and achieve complete degradation of DEHP (50 mg/L) in 48 h. The kinetics of DEHP degradation by RL-HY01 were well fitted with the modified Gompertz model. The metabolic intermediates of DEHP by strain RL-HY01 were identified by ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis and then the metabolic pathway of DEHP was deduced. DEHP was transformed into di-ethyl phthalate (DEP) via β-oxidation and then DEP was hydrolyzed into phthalic acid (PA) by de-esterification. PA was further transformed into gentisate via salicylic acid and further utilized for cell growth. Bioaugmentation of strain RL-HY01 with marine samples was performed to evaluate its application potential and the results suggested that strain RL-HY01 could accelerate the elimination of DEHP in marine samples. The results have advanced our understanding of the fate of PAEs in marine ecosystem and identified an efficient bioremediation strategy for PAEs-polluted marine sites.

Keywords: Marine microbe; Phthalic acid esters;, Metabolic kinetics; *Mycolicibacterium phocaicum*; Bioaugmentation

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1. Introduction

The increasing discharge of plastic debris into the marine environment has drawn worldwide attention (Thompson et al., 2004), especially the formation of microplastic particles (Ahmed et al., 2021). As one of the main destinations of plastic wastes, plastics are ubiquitous in marine ecosystem and it was estimated that 4.8 to 12.7 million tons of plastic waste entered the oceans in 2010 (Jambeck et al., 2015). Plastics entering the oceans will eventually be decomposed into microplastic debris by mechanical abrasion, or photodecomposition (Andrady, 2005). Marine plastics pose huge threats to ocean ecosystem during this process because of the release and sorption of contaminants. When plastic debris are decomposed into microplastics (MPs), they might adsorb some other pollutants (such as heavy metals, PAHs, PCBs, etc.) and become the vector for transportation, which might be ingested by marine organisms and make the carried contaminants to be accumulated in food chain (Brennecke et al., 2016; Devriese et al., 2017; Lucia et al., 2018). Meanwhile, some eco-toxic plastic additives like plasticizers and flame retardants would be released from polymers during the decomposition, which might also pose great threats to marine organisms (Hermabessiere et al., 2017; Koelmans et al., 2014). Although marine plastic pollution has attracted great concerns and has become a pervasive global environmental issue, the investigations are mainly about the toxic evaluation, distribution, related environmental policy, et al. However, the fate of the released plastic additives in marine ecosystem has not been well studied.

Plasticizers are ubiquitously added to plastic polymers to improve their flexibility and durability. Plasticizers can make up for 10 to 70% of plastics' weight (Wright et al., 2020). As the most widely used plasticizer, di-(2-ethylhexyl) phthalate (DEHP, one kind of PAEs) makes up the majority of plasticizer market (Ren et al., 2018). It is estimated that the annual consumption of DEHP is above 3 million tons globally. Since DEHP is a typical external plasticizer, it could be released into environments during the manufacturing, using and disposing of plastics (Schiedek, 1995). Because of marine plastic pollution, DEHP has been detected in all oceans (Paluselli and Kim, 2020; Lubecki and Kowalewska, 2019; Zhang et al., 2019; Savoca et al., 2018). For the above reasons, great concerns on the safety of DEHP have been raised and related investigations have classified DEHP as endocrine disruptor and carcinogen. The origin, distribution, transformation, and fate of DEHP in terrestrial ecosystems have been systematically investigated. However, the knowledge on the fate of DEHP in marine ecosystem is still limited.

The transformation of pollutants in different environments is considered to be the mass balance-wise main route of matter cycle (Fenner et al., 2013). Since photolysis and chemical hydrolysis of PAEs are relatively slow, microbes-mediated degradation of PAEs is known as the major route for natural decomposition of PAEs in different ecosystem (Baker et al., 2021; Zhao et al., 2019; Cheng et al., 2018). Though lots of PAEs-degrading microbes have been isolated and characterized, marine PAEs-degrading microbes are limited (Wright et al., 2020). The metabolic pathways and related molecular mechanisms of PAEs biodegradation have been extensively investigated, mainly in terrestrial microorganisms (Xu et al., 2021; Wang et al., 2019; Yang et al., 2018; Zhao et al., 2018; Nahurira et al., 2017). The typical metabolic pathways of PAEs include two main steps: (a) transformation of PAEs into phthalic acid (PA), and (b) utilization of PA. In some isolates, long side-chain PAEs (e.g., DEHP) were converted into short side-chain PAEs (e.g., di-ethyl phthalate (DEP) or di-methyl phthalate (DMP)) prior to de-esterification via β-oxidation and demethylation (Ren et al., 2018). As to the utilization of PA, it was commonly transformed into protocatechuate and further utilized through protocatechuate branch of β-ketoadipate pathway. Several enzymes involved in the hydrolysis of ester bonds in PAEs have been identified and related catalytic mechanisms have been characterized to some extent in a number of terrestrial isolates. Nevertheless, previous studies mainly focused on biodegradation by terrestrial microorganisms, while

45 the degradation of PAEs in the marine ecosystem remains underexplored.

46   The aims of this study were to isolate and characterize PAEs-degrading marine bacterial strain from

47 intertidal sediments. One DEHP-degrading *Mycolicibacterium phocaicum* strain RL-HY01 was isolated

48 with the capability of utilizing several kinds of PAEs as sole carbon source for growth. Strain RL-HY01

49 showed good adaptability to different environmental factors. Tween-80 could accelerate the degradation

50 by enhancing the solubility of DEHP and its kinetics followed the modified Gompertz model. The

51 metabolic pathway of DEHP was deduced by metabolic intermediates identification. Bioaugmentation

52 of strain RL-HY01 with synthetic DEHP-contaminated marine samples was performed to evaluate its

53 application potential.

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55 **2. Materials and methods**

56 **2.1. Chemicals, medium and culture conditions**

57   The standards of DEHP (98% purity), di-cyclohexyl phthalate (DCHP, 99% purity), di-butyl

58 phthalate (DBP, 99% purity), di-ethyl phthalate (DEP, 99% purity), di-methyl phthalate (DMP, 99.5%

59 purity) were purchased from J&K Scientific (Beijing, China). The stock solution of PAE with the

60 concentration of 2×10^4 mg/L was prepared with methanol. Tween-80, Brij-35 and Triton X-100 were

61 obtained from Sangon Biotech (Shanghai, China). Acetonitrile and *n*-hexane of HPLC grade were

62 purchased from Sigma-Aldrich. Enzymes and biological reagents were obtained from Takara (Japan).

63 Reagents for the preparation of microbial cultivation medium and all other chemicals were of analytical

64 grade and purchased from Sinopharm Chemical Reagent (Shanghai, China).

65   The isolation of DEHP-degrading bacterial strain and tests of degrading capacity were conducted

66 in mineral salt medium (MSM) with the composition per liter of: NaCl (20 g), $NaH_2PO_4 \cdot 12H_2O$ (4.8 g),

67 K_2HPO_4 (3.6 g), KH_2PO_4 (4.5 g), $(NH_4)_2SO_4$ (1.2 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), $FeCl_2$ (0.05 g), and $CaCl_2$

68 (0.03 g), at pH 7.0. The stock solution of PAE was added into the sterilized MSM to obtain the target

69 concentration which served as the sole carbon resource. The bacterial inoculum was prepared in modified

70 Luria-Bertani (LB) medium consisted of: peptone (10 g), yeast extract (5 g), and NaCl (25 g), per liter,

71 at pH7.0. The solid media of MSM and LB were prepared by adding agar with a final concentration of

72 15 g/L. All media were sterilized by autoclaving at 121 ℃ for 30 minutes.

73 **2.2. Enrichments and Microbial Isolation from intertidal sediments**

74   Intertidal sediment samples were collected from the intertidal of Zhanjiang Bay, China. The detailed

75 information of these samples is presented in Table S1. Approximately 5 g of sediment sample was

76 inoculated into 50 mL of LB medium and supplemented with 50 mg/L of DEHP. The enriched cultures

77 incubated (180 rpm) at 30 ℃ for 5 days. And then 1 mL of enrichment suspension was transferred into

78 10 mL of fresh LB medium and the concentration of DEHP was adjusted to 100 mg/L. The steps for

79 enrichment were repeated until the concentration of DEHP was increased to 500 mg/L. The final

80 enrichment suspension was spread onto the MSM solid medium supplied with 500 mg/L of DEHP and

81 0.01 g/L of Tween 80. Well grown colonies with hydrolytic halos were further selected for confirmation.

82 Briefly, (a) potential single colony was inoculated into fresh LB medium and incubated (180 rpm) at

83 30 ℃ for 24 hours, (b) cells in 1 mL of cultures were harvested by centrifugation (6000 rpm, 2 minutes),

84 washed by phosphate buffer solution (PBS, pH 7.8) and re-centrifugated (6000 rpm, 2 minutes) for three

85 times, (c) the cell pellets were finally resuspended in 1 mL of fresh MSM liquid medium and inoculated

86 into 9 mL of MSM supplemented with 50 mg/L of DEHP, and (d) cultures were incubated (180 rpm) at

87 30 ℃ for 5 days and then used for the determination of DEHP concentration. Steps (a) to (d) were

88 repeated until stable DEHP-degrading strain was isolated.

2.3. Biochemical and 16S rRNA gene identification of DEHP-degrading strain

The identification of isolated DEHP-degrading strain was accomplished by 16S rRNA gene analysis coupled with biochemical characterization. The amplification, sequencing and analyzing of 16S rRNA gene were conducted prior to biochemical characterization. The amplification of 16S rRNA gene was achieved by polymerase chain reaction (PCR) with universal primers of 27F and 1492R. The amplified fragments were inserted into the p-MD19T vector and then sequenced by Thermo Fisher Scientific (Guangzhou, China). VecScreen (https://www.ncbi.nlm.nih.gov/tools/vecscreen/) was used for removing the vector fragments from the obtained sequences and the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) was employed to identify the resulting 16S rRNA gene sequences. According to the BLASTN search results, 16S rRNA genes of related type strains were retrieved from the List of Prokaryotic names with Standing in Nomenclature (LPSN, http://www.bacterio.net/) for phylogenetic analysis (Parte, 2018). Phylogenetic analysis was accomplished via MEGA 7.0 with a Neighbor-Joining algorithm (bootstrap value was 1000) (Kumar et al., 2016). According to phylogenetic analysis results, the biochemical characteristics of isolated strain and its closest species was compared as referenced in Bergey's Manual of Determinative Bacteriology (George et al., 2001).

2.4. Characterization of isolated strain

The seeds for inoculum were prepared by inoculating the single colony of isolated strain into 10 mL of LB and incubating (180 rpm) at 30 °C for 24 hours. Bacterial cells in 1mL of the culture for the following assays were harvested by centrifugation (8000 rpm, 3 min) and the cell pellets were washed by PBS buffer (pH 7.8, 100 mM). The centrifugation and washing were repeated for three times, and the cells were resuspended in 1 mL of fresh MSM. The cell density of the obtained inoculum was approximately 7.0×10^7 cells/mL with OD_{600} of 0.8. The inoculation ration for the following assays was maintained at 1.0% (v/v) unless stated otherwise.

The isolated strain was inoculated in 50 mL Erlenmeyer's flasks containing 10 mL of MSM supplemented with 50 mg/L DEHP. The cell growth (OD_{600}) and the residual concentration of DEHP were measured with an interval of 12 h during 72 h. The capacity for utilizing other PAEs for growth was detected by adding DCHP, DBP, DEP and DMP as sole carbon sources separately, and the effect of PAEs concentrations (500 mg/L, 700 mg/L, 1000 mg/L, 1200 mg/L, and 1500 mg/L) on biodegradation was measured. The degradation of selected PAEs under different pH (4, 5, 6, 7, 8, 9, and 10), temperature (10 °C, 20 °C, 30 °C, 40 °C, and 50 °C), and salinity (4.0%, 6.0%, 8.0%, 10.0% and 12.0%, w/v). The control treatments for the above assays were performed in same cultures without inoculation and incubated under the same conditions. Samples were withdrawn after 72 hours' incubation and subsequently applied to the measurements of substrate concentration. The residual concentration of PAEs was detected by gas chromatography (GC). All assays were performed in triplicate.

Since the selected PAEs are insoluble in water, their real concentrations in MSM are relatively low. Therefore, nonionic surfactants were used to improve the solubility of DEHP and its effects on the growth of isolated DEHP-degrading bacterial strain was evaluated as well as the influence on DEHP degradation. The detailed information of selected nonionic surfactants was presented in Table S2. The improvement of DEHP solubility was measured using a modified method from previous report (Navacharoen and Vangnai, 2011). Briefly, (a) standard solution was prepared by adding DEHP into fresh MSM to a theoretic concentration of 100 mg/L, (b) different surfactants were added into the prepared standard solution (50 mL) in Erlenmeyer's flasks with different multiples (0, 0.1, 0.5, 1, 2) of critical micellar concentration (CMC), separately, (c) the obtained mixtures were equilibrated on a rotary shaker at 120

rpm for 24 h, and (d) 20 mL of the mixture below the surface layer was withdrawn for DEHP concentration determination.

Further, the effects of selected surfactants on cell growth were evaluated. DEHP-degrading bacterial strain was inoculated into fresh MSM in the presence of each surfactants (optimized minimum concentration) and glucose (50 mg/L) was supplemented as carbon resources. Cultures were incubated (180 rpm) at 30 °C for 24 hours and then the cell concentration (OD_{600}) was determined. Finally, the surfactant that showed the lowest adverse effects on cell growth was selected for the following assays. Firstly, the biodegradation of DEHP by the isolated strain was measured with and without the selected surfactant. The concentration of surfactant was added with the optimized minimum concentration. The residual concentration of DEHP was determined at every 12 h intervals. And then, a modified Gompertz model (Eq. 1) and a first-order decay model (Eq. 2) were applied to simulate the kinetics of DEHP degradation with and without the supplementation of surfactant (Zhang et al., 2020).

The modified Gompertz model: $S = S_0 - A \cdot \exp\left\{-\exp\left[\frac{Vm \cdot e}{A} \cdot (L - t) + 1\right]\right\}$ (1)

The first−order decay model: $S = S_0 + A \cdot \exp(-t/t_1)$ (2)

In which S represents the substrate concentration; S_0 represents the fitted initial concentration; A is the biodegradation potential; Vm means the maximum biodegradation rate; and L represents the lag phase.

2.5. Metabolites and metabolic pathways of DEHP

The isolated DEHP-degrading strain was inoculated in MSM (containing 50 mg/L DEHP), incubated and sampled as described above. Filtered supernatants (0.45 μm) were applied for metabolites extraction and two steps extraction was used. Briefly, (a) equal volume of *n*-hexane was added into the filtered supernatant and fully extracted by ultrasonic extraction for 10 min after which the organic portion was collected as neutral extracts, and (b) the rest aqueous portion was adjusted to pH 3.0 using 2 M HCl and then extracted as described in step (a) in which the extracts were classified as acid extracts. And then, the neutral extracts and acid extracts were merged, evaporated, redissolved in methanol and subsequently applied for metabolites identification. Finally, the metabolic pathways of DEHP in the isolated strain were deduced according to the identified metabolites.

2.6. Bioaugmentation with synthetic DEHP-contaminated marine samples

Since the isolation and application of marine DEHP-degrading strains are rarely reported, the bioaugmentation of isolated DEHP-degrading strain into synthetic DEHP-contaminated marine samples was conducted and the degrading capacity of DEHP was evaluated. Seawater and intertidal sediments were collected from Naozhou island (Zhanjiang, China). The characteristics of marine samples are presented in Table S1. For the seawater, the seeds of isolated strain were inoculated in 50 mL glass vials containing 10 mL of seawater supplemented with 50 mg/L DEHP with an inoculum ration of 1% (*v/v*). Seawater (containing 50 mg/L DEHP) without inoculation was set as the control treatment. Three types of intertidal sediments (Mud, M; Sand, S; Mud and sand mixed, MS) were sampled and applied for the following assays. For the intertidal sediments, the bioaugmentation was conducted in a glass beaker with 20 g sediments. DEHP was added to the sediments with a final concentration of 50 mg/kg and 2 mL of bacterial seeds were inoculated. Sediments (containing 50 mg/kg DEHP, all on dry weight basis?) without inoculation was set as the control treatment. All samples were fully mixed and all treatments were conducted in triplicate. Seawater samples were incubated under constant shaking (80 rpm) at 30 °C while sediment samples were incubated under constant temperature (30 °C) and humidity (90% relative humidity). DEHP in seawater was extracted as described above and DEHP in sediments was extracted according previous reports (Zhang et al., 2020). The residual concentration of DEHP in sediments was

176 measured with an interval of 3 d during 21 d while the time interval for seawater was 12 h during 72 h.

177 **2.7. Analytic methods**

178 A GC system (GC-2010 pro, SHIMADZU, Japan) equipped with a WondaCap 5 column (GL

179 Sciences Inc., Japan, 30 m×0.25 mm×0.25 μm) and electron capture detector (ECD) was used for

180 quantification of selected PAEs. An oven temperature program of 100 °C for 5 min, increased to 260 °C

181 with a rate of 20 °C/min, and finally maintained at 260 °C for 15 min was used for the quantitative

182 detection while the temperatures of inlet and detector were maintained at 300 °C and 280 °C, respectively.

183 The injection volume was 2 μL and nitrogen (purity > 99.999%) was used as the carrier gas with a flow

184 rate of 2 mL/min. Data acquisition and analysis was executed on LabSolutions (version 5.90, Shimadzu,

185 Japan). The standard curves of selected PAEs were established and presented in Table S3. DEHP in

186 marine samples was extracted by n-hexane, dried by nitrogen blow, and redissolved in methanol.

187 Membrane filtering of all samples was conducted prior to GC analysis. The recovery rates of DEHP were

188 measured with the average recovery rate of 96.8% (all above 94.0%). The degradation percentages of

189 PAEs were calculated with Eq. (3):

190 Degradation percentage (%) = $(C_{ckf} - C_f)/C_{ckf} \times 100$ (3)

191 where C_{ckf} means the final concentration of PAE in control treatment and C_f represents the final

192 concentration of PAE in the target treatment.

193 The cell growth was monitored by the absorbance at 600 nm (OD_{600}). Cells in collected cultures

194 were harvested by centrifugation (6000 rpm, 5 min), washed by PBS buffer (pH 7.8), and the

195 centrifugation and washing were repeated three times. Finally, cell pellets were resuspended in PBS

196 buffer (equal to initial volume) and then the cell density was measured by a spectrophotometer

197 (MAPADA, P4PC, China).

198 The identification analysis of DEHP metabolites was accomplished by ultra-performance liquid

199 chromatography coupled with mass spectrometry conducted by Bionovogene Co. Ltd (Suzhou, China).

200 Chromatographic separation was accomplished in an Thermo Ultimate 3000 system equipped with an

201 ACQUITY UPLC HSS T3 (150×2.1 mm, 1.8 μm, Waters) column maintained at 40 °C. The temperature

202 of the autosampler was 8 °C. Gradient elution of analytes was carried out with 0.1% formic acid in water

203 (A) and 0.1% formic acid in acetonitrile (B) or 5 mM ammonium formate in water (C) and acetonitrile

204 (D) at a flow rate of 0.25 mL/min. Injection of 2 μL of each sample was done after equilibration. An

205 increasing linear gradient of solvent B (v/v) was used as follows: 0~1 min, 2% B/D; 1~9 min, 2%~50%

206 B/D; 9~12 min, 50%~98% B/D; 12~13.5 min, 98% B/D; 13.5~14 min, 98%~2% B/D; 14~20 min, 2%

207 B-positive model (14~17 min, 2% D-negative model). The ESI-MSn experiments were executed on the

208 Thermo Q Exactive Focus mass spectrometer with the spray voltage of 3.8 kV and -2.5 kV in positive

209 and negative modes, respectively. Sheath gas and auxiliary gas were set at 30 and 10 arbitrary units,

210 respectively. The capillary temperature was 325 °C. The analyzer scanned over a mass range of m/z 81-

211 1 000 for full scan at a mass resolution of 70 000. Data dependent acquisition (DDA) MS/MS

212 experiments were performed with HCD scan. The normalized collision energy was 30 eV. Dynamic

213 exclusion was implemented to remove some unnecessary information in MS/MS spectra. The entire

214 process of metabolites characterization and annotation was based on known MS/MS database (Table S4).

215 The processing, visualization and analysis of mass spectrometry based molecular profile data was

216 executed on MZmine 2 (version 2.53) (Pluskal et al., 2010).

217 **2.8. Accession numbers**

218 Strain RL-HY01 is available from Guangdong Microbial Culture Collection Center (GDMCC) with

219 accession number of 61246. The 16S rRNA gene of strain RL-HY01 is accessible in GenBank with

220   accession number MK787328.

221

222   **3. Results and discussion**

223   **3.1. Isolation and identification of DEHP-degrading marine bacterial strain**

224   The sampling intertidal sites are around a municipal wastewater treatment outlet. An isolated

225   bacterial strain, RL-HY01 could completely degrade 50 mg/L of DEHP in 72 hours with significant cell

226   growth. The 16S rRNA gene of strain RL-HY01 with a length of 1505 bp was amplified and subsequently

227   applied to BLAST search. Phylogenetic analysis of strain RL-HY01 with related type strains were

228   performed and the result indicated that strain RL-HY01 was clustered with *Mycolicibacterium*

229   *phocaicum* CIP 108542[T] (Figure 1). The physiological and biochemical characteristics of strain RL-

230   HY01 were presented in Table 1. Finally, strain RL-HY01 was identified as *Mycolicibacterium*

231   *phocaicum* according to 16S rRNA gene analysis, and its physiological and biochemical characteristics.

232   Genus *Mycolicibacterium*, previously known as the members of genus *Mycobacterium*, including

233   91 species now (Yamada et al., 2018). Genus *Mycobacterium* is known as decomposer of xenobiotics

234   that has been isolated from various environments (Johnston et al., 2017; Kandil et al., 2015; Child et al.,

235   2007; Sutherland et al., 2002). However, limited isolates of genus *Mycolicibacterium* (or *Mycobacterium*)

236   capable of degrading PAEs were reported and the related information was summarized in Table 2. Strain

237   NK0301 was the first reported *Mycobcterium* capable of degrading PAEs (Nakamiya et al., 2005). Strain

238   NK0301 could degrade DEHP into 2-ethylhexanol and 1,2-benzenedicarboxylic acid, and it could

239   remove up to 90% of DEHP in polyvinyl chloride sheets within 3 d. *Mycobacterium* sp. YC-RL4 is a

240   DEHP-degrading strain isolated from petroleum-contaminated soil which could utilize a wide range of

241   PAEs as sole carbon source for growth. Strain YC-RL4 could transform DEHP into PA via MEHP and

242   PA was further utilized for growth via benzoic acid (BA) degradation pathway (Ren et al., 2016).

243   Interestingly, strain YC-RL4 could adjust its cell surface hydrophobicity when incubated with DEHP

244   which could be beneficial for biodegradation by increasing the accessibility of the hydrophobic DEHP.

245   Strain DBP42, the sole reported marine PAEs-degrading *Mycobacterium* strain, was isolated from marine

246   plastic debris (Wright et al., 2020). Strain DBP42 could degrade a wide range of PAEs by transforming

247   PAEs into PA via DAPs and utilizing of PA via protocatechuate branch of β-ketoadipate pathway.

248   Furthermore, the molecular mechanism of DEHP degradation in *Mycobacterium* spp. is still unknown

249   and it is not known whether the mechanism for biodegradation is the same for terrestrial and marine

250   *Mycobacterium* isolates. Hence, further isolation of PAEs degrading *Mycobacterium* strains and

251   investigation of related mechanisms are needed.

252   **3.2. Characterization of *Mycolicibacterium phocaicum* RL-HY01**

253   Since plasticizer PAEs includes dozens of compounds, the investigation of degrading capability of

254   other representative PAEs became necessary. All the selected PAEs except DCHP could be utilized by

255   strain RL-HY01 as sole carbon source with significant cell growth. The substrate profile analysis

256   indicated that strain RL-HY01 could utilize linear side chain and branched side chain PAEs, but failed to

257   utilize ring side chain PAEs. Further, the degradation percentages of selected PAEs with different initial

258   concentrations were measured after 72 hours' incubation (Figure 2A). When the substrate concentration

259   was below 1000 mg/L, the degradation percentages of all selected PAEs were 100% while the

260   degradation and cell growth were significantly inhibited when substrate concentration was increased to

261   1500 mg/L. Strain RL-HY01 could degrade the selected PAEs under a wide range of pH (Figure 2B).

262   The degradation percentages were all above 60% under pH 5.0 to 9.0 while no cell growth and limited

263   degradation of PAEs were observed when pH were 4.0 and 10.0. The degradation of DEHP was

264 significantly inhibited when incubation temperature was too low (10 ℃) or too high (50 ℃) while strain
265 RL-HY01 showed a good performance when the incubation temperature was ranged from 20 ℃ to 40 ℃
266 (Figure 2C). In addition, the optimal temperature for degradation was 30 ℃, in which all the selected
267 PAEs were completely degraded. Since the salinity of MSM was around 3% during enrichment and
268 isolation, the investigation of the effects of salinity on PAEs degradation was conducted with salinity
269 ranged from 4% to 12% (Figure 2D). When the salinity was around 4% to 8%, the selected PAEs were
270 completely degraded within 72 h and the degradation percentage of PAEs decreased significantly when
271 the salinity was increased to 12%. The substrate profile reflects the potential metabolic pathways in
272 microbes which were determined by the intrinsic molecular mechanisms. Strain RL-HY01 failed to
273 utilize DCHP, which suggested that the enzymes in strain RL-HY01 could not work on PAEs with closed
274 circular chain. As to the known PAE-degrading *Mycobacterium* spp., only *Mycobacterium* sp. YC-RL4,
275 a terrestrial bacterial strain, is able to degrade DCHP, indicated that the metabolic mechanisms of PAEs
276 between terrestrial and marine microbes might be different (Ren et al., 2016). The underlying reason
277 could be illuminated by analyzing the molecular mechanisms involved in the metabolism of other PAEs.
278 As to the effects of environmental factors, strain RL-HY01 exhibited good tolerance to a wide range of
279 pH, temperature, and salinity. Specifically, strain RL-HY01 could efficiently degrade DEHP under high
280 salinity, with 72.8% of DEHP degraded under salinity 10.0% within 72 h. The good performance of strain
281 RL-HY01 under high salinity might be due to high salinity of intertidal sediments where strain RL-HY01
282 was isolated. The tolerance of high salinity would be a key characteristic for industrial application and
283 marine microbes are known to be important resources for industrial development.

284 　　The addition of surfactant has been proved to be able to promote the solubility of hydrophobic
285 compounds (Paria, 2008). The detected concentrations of DEHP with the addition of different
286 concentration of surfactants were shown in Figure 3A. The concentration of DEHP in aqueous phase
287 increased with the increasing of surfactants concentration which could reach its maximum concentration
288 (viz. added concentration). However, the requirements of surfactants' concentration were different for
289 the target maximum concentration of DEHP and they were half CMC of Tween 80, and 1×CMC of Brij-
290 35 and Triton-X-100, respectively. Thus, half CMC of Tween 80, and 1×CMC of Brij-35 and Triton-X-
291 100 were selected for the further tests of cell toxicity. As shown in Figure 3B, all the selected
292 concentration of surfactants showed toxic effects on the growth of strain RL-HY01 of which Brij-35 and
293 Triton-X-100 significantly inhibited the cell growth while Tween 80 showed the least toxic effect. Since
294 Tween 80 yielded highest solubilization of DEHP with relative lower concentration (0.5×CMC) and
295 showed least toxic effects to strain RL-HY01, it was selected as the solubilizing agent to establish a stable
296 homogeneous system for the modeling of degradation kinetics.

297 　　The degradation of DEHP by strain RL-HY01 when supplemented with or without Tween 80 is
298 presented in Figure 3C. When Tween 80 was added, the degradation was accelerated and 50 mg/L was
299 completely degraded within 48 h while 60 h was needed for the treatment without Tween 80. Further, the
300 obtained residual concentrations of DEHP in both treatments were applied for the modeling of
301 degradation kinetics with a modified Gompertz model and a first-order decay model. The results revealed
302 that the degradation of DEHP by strain RL-HY01 fitted with the modified Gompertz model better,
303 compared with the first-order decay model (Table 3). Meanwhile, the kinetics analysis indicated that
304 DEHP biodegradation by strain RL-HY01 supplemented with Tween-80 followed the modified
305 Gompertz model with a higher correlation coefficient (R^2=0.9998) than DEHP biodegradation by strain
306 RL-HY01 without the supplementation of Tween-80 (R^2=0.9857). The best-fitted curves for DEHP
307 degradation by strain RL-HY01 using the modified Gompertz model are presented in Fig. 3D and the

308 kinetic parameters were shown in Table 3. When supplemented with Tween-80, the degradation was
309 promoted with a higher DEHP biodegradation rate (2.7 mg/L/h) and shorter lag phase time (10.7 h),
310 compared with DEHP degradation without supplementation of Tween-80 (1.30 mg/L/h and 22.19 h,
311 respectively). The results suggested that strain RL-HY01 performed better with the supplementation of
312 Tween-80 and the enhanced solubility of DEHP by Tween-80 might be the potential reason.

313 Knowledge of the biodegradation kinetics and their dependency on environmental factors might
314 provide key insights into the understanding of the fate and transformation of substrates in many
315 environmental systems. A multitude of factors might affect microbial degradation kinetics in a particular
316 environmental system, but the substrate abundance is known to be the most dominant effect among all
317 (Gharasoo et al., 2015). For the degradation of xenobiotics, a high substrate concentration might have
318 toxic effects and therefore inhibit the degradation process, while the bioavailability limitations of some
319 insoluble pollutants (such as DEHP) can therefore promote the degradation by softening the contaminant
320 toxicity effects to microbes (Gharasoo et al., 2015; Semple et al., 2004). However, the low concentration
321 could also slow down the biodegradation by reducing the microbial access to the substrate (Bosma et al.,
322 1996). Thus, the degradation could be promoted by improving the accessibility of hydrophobic
323 compounds to a suitable range. Generally, there are two strategies for microbes to improve the
324 bioavailability of such kind of compounds, (i) adjust the cell properties of microbes, for example,
325 *Mycobacterium* sp. YC-RL4, a PAEs-degrading bacterium, could adjust its cell surface hydrophobicity
326 to improve the bioavailability of PAEs, which has been proved to be able to accelerate the biodegradation
327 (Ren et al., 2016), and (ii) produce some cosolvent (e.g., biosurfactant) to promote the solubility of
328 hydrophobic compounds as well as the synthetic surfactants (Patowary et al., 2017; Wu et al., 2019).
329 Numerous of biosurfactant producing and hydrophobic compounds degrading bacterial strains have been
330 isolated and they were proved to be an efficient approach for elimination of hydrophobic compounds. In
331 addition, the toxicity of biosurfactants is commonly less than synthetic surfactants while the
332 solubilization effects of biosurfactants is generally better than synthetic surfactants. However, the
333 application of biosurfactants is limited for their low production. Although the synthetic surfactants are
334 known to be toxic to microbes, they were extensively applied in the biodegradation of hydrophobic
335 compounds because of their low-costs and favorable stability. *Bacillus subtilis* strain 3C3, an organic-
336 solvent-tolerant and PAEs degrading bacterium, showed better performance in the presence of surfactant
337 (Tween-80) during the degradation of PAEs (Navacharoen and Vangnai, 2011). For the isolated DBP-
338 degrading soil bacteria, six surfactants were tested for their abilities to increase degradation rate in the
339 isolated DBP-degrading strains and all of them showed growth inhibition against the tested strains(Chao
340 et al., 2006). Further study found that soil could minimize surfactant toxicity of surfactant and increase
341 the degradation potential of some of the test bacteria. It was also found that the effects of surfactants and
342 the requirement of surfactant's concentration varied with bacterial species (Chao et al., 2006; Allen et
343 al., 1999). For our investigation, it can be concluded that strain RL-HY01 showed good tolerance with
344 Tween-80 and better DEHP degradation performance in the presence of Tween-80.

3.3. Metabolites and metabolic pathways of DEHP

346 The metabolic pathways (Figure 4A) of DEHP in strain RL-HY01 was proposed through metabolic
347 intermediates identification. Apart from the parent compound DEHP, several compounds were detected
348 (Figure 4B) and then MS/MS analysis was executed. For identification of some compounds, the RT and
349 MS/MS results were both taken into account to distinguish the compounds share same molecular weights.
350 According to the MS/MS results (Figure S1), di-*n*-hexyl phthalate (D*n*HP), di-(2-ethylbutyl) phthalate
351 (DEBP), DBP, DEP, PA, salicylic acid and gentisic acid were identified from the extracts. All these

metabolites disappeared after 72 h incubation, which suggested that DEHP was completely degraded by strain RL-HY01. DEHP was transformed into shorter side chain PAEs (DnHP, DEBP, DBP, and DEP) step by step via β-oxidation in strain RL-HY01. Subsequently, DEP was hydrolyzed into PA and then PA was transformed into gentisic acid via salicylic acid. Finally, gentisic acid was exploited for cell growth through β-ketoadipate pathway. For the transformation of PAEs into PA, β-oxidation in prior of de-esterification is better than direct de-esterification since some released side chains are recalcitrant to degradation and toxic to environmental organisms. These processes might also explain why the degradation of shorter side-chain PAEs (DMP) is faster than the longer side-chain PAEs (e.g. DEHP) by strain RL-HY01 although the difference is not significant. Meanwhile, the requirements for the transformation of DEHP into DEP by β-oxidation in strain RL-HY01 could also explain why strain RL-HY01 failed to utilize DCHP (circle side-chain). In addition, the simultaneous detection of DnHP and DEBP indicated that there is no special order for the β-oxidation. As to the utilization of PA, the metabolic pathway showed some differences with most reported PAEs-degrading microbes. As we know, most of the isolated PAEs degrading microbes transformed PA into protocatechuic acid which was further utilized via the protocatechuate branch of the β-ketoadipate pathway (Ren et al., 2018). Although salicylic acid and gentisic acid have been identified during PAEs biodegradation in a few of recent reports (Yu et al., 2020), the recognition of the complete metabolic pathway and related molecular mechanism of the gentisate branch of the β-ketoadipate pathway in PAEs-degrading bacteria was underrepresented. According to our knowledge, this is the first report of gentisate branch of the β-ketoadipate pathway mediated PAEs degradation in marine microbe which might provide novel insights into the metabolic diversity of PAEs in marine ecosystem.

3.4. Bioaugmentation with synthetic DEHP-contaminated marine samples

The degradation of DEHP in the synthetic DEHP-contaminated marine sample was presented in Figure 5. The degradation of DEHP in seawater by strain RL-HY01 showed similar tendency with the performance in MSM and the natural degradation of DEHP in seawater was very slow which can be neglected. The performance of strain RL-HY01 in seawater indicated that strain RL-HY01 could be a robust candidate for the bioremediation of PAEs-contaminated seawater. The degradation of DEHP in intertidal sediments showed some differences with different types of sediments. Firstly, the natural degradation percentages of DEHP (without bioaugmentation of strain RL-HY01) in muddy sediment, sandy sediment and mixed sediment were 5.7%, 2.1%, and 8.9%, respectively. The difference of natural degradation percentages of DEHP in different sediments indicated that different types of sediments possess different degradation potential towards PAEs. As we know, the microbial diversity and abundance in sand are relatively low as well as the nutrition which are essential for the microbe-mediated elimination of xenobiotics. Further, the natural degradation of DEHP in muddy and mixed sediments might be mediated by the natural hydrolysis or potential indigenous marine microbes. Secondly, the degradation percentages of DEHP in the bioaugmented muddy sediment, sandy sediment and mixed sediment were 57.6%, 79.3%, and 92.5%, respectively. Although we know that the elimination of pollutants in environments is predominated by microbes, the process of microbe mediated biodegradation is affected by the condition of the habitat, such as environmental pH, oxidant, indigenous microbes, and sunlight irradiation. In this study, different types of sediments represent different environmental matrices which possess different reaction conditions for the bioaugmented strain. Muddy sediment is more likely to form an anaerobic environment which may have negative effect on the degradation of DEHP by strain RL-HY01. Meanwhile, muddy sediment contains numerous amounts of organic matter which may adsorb some hydrophobic DEHP and therefore decreases its bioavailability. As to the sandy sediment,

the lack of indigenous microbe and nutrition could be important limitations for the elimination of DEHP by strain RL-HY01. However, the degradation percentage of DEHP in sandy sediment is still higher than the one in muddy sediment and this suggests that oxygen could be an important condition for DEHP biodegradation. The degradation percentage of DEHP in the mud and sand mixed sediment is the highest which might be contributed by the potential indigenous degraders, good environmental factors (nutrition and oxygen), and well adaptability of strain RL-HY01. In general, the bioaugmentation of strain RL-HY01 with marine sample demonstrated that strain RL-HY01 could be used as a potential and efficient PAEs degrader for the bioremediation of contaminated marine sites.

4. Conclusion

In this study, one new marine bacterial strain RL-HY01 with high DEHP degradation ability was isolated from intertidal sediments polluted by municipal wastewater, and systematically identified and characterized. Strain RL-HY01 showed good environment adaptability and Tween-80 was found to be able to promote the biodegradation. The kinetics analysis of DEHP degradation by strain RL-HY01 indicated that the process followed the modified Gompertz model well (R^2=0.9998). The metabolic intermediates of DEHP in strain RL-HY01 were identified by UHPLC-MS/MS and the metabolic pathway was proposed. DEHP was transformed into PA by β-oxidation and de-esterification which was further utilized by gentisate branch of the β-ketoadipate pathway, which has not been reported in PAEs-degrading isolates. Finally, the bioaugmentation of strain RL-HY01 with synthetic DEHP-contaminated marine samples was performed and strain RL-HY01 could remove DEHP from these samples efficiently, with a maximum DEHP degradation percentage of 92.5 (50 mg/kg, 21 d). These results might advance our understanding about the fate of PAEs in marine ecosystem and also provide an efficient degrader for developing bioremediation strategies for PAEs-polluted marine sites.

References

Ahmed, M.B., Rahman, M.S., Alom, J., Hasan, M.S., Johir, M.A.H., Mondal, M.I.H., Lee, D., Park, J., Zhou, J.L., Yoon, M., 2021. Microplastic particles in the aquatic environment: a systematic review. Sci. Total Environ. 775, 145793.

Allen, C.C.R., Boyd, D.R., Hempenstall, F., Larkin, M.J., Sharma, N.D., 1999. Contrasting effects of a nonionic surfactant on the biotransformation of polycyclic aromatic hydrocarbons to cis-dihydrodiols by soil bacteria. Appl. Environ. Microb., 65(3), 1335-1339.

Andrady, A.L., 2005. Plastics and the environment. John Wiley & Sons.

Baker, A., Ahmad, B., Alarjani, K.M., Aldosri, N.S., Khan, M.S., 2021. Biostimulation of *Rhodovulum* sp., for enhanced degradation of di-n-butyl phthalate under optimum conditions. Chemosphere, 266, 128998.

440   Bosma, T.N.P., Middeldorp, P.J.M., Schraa, G., Zehnder, A.J.B., 1996. Mass transfer limitation of
441   biotransformation: quantifying bioavailability. Environ. Sci. Technol., 31(1), 248-252.

442   Brennecke, D., Duarte, B., Paiva, F., Caçador, I., Canning-Clode, J., 2016. Microplastics as vector for
443   heavy metal contamination from the marine environment. Estuar. Coast. Shelf S., 178, 189-195.

444   Chao, W.L., Lin, C.M., Shiung, I.I., Kuo, Y.L., 2006. Degradation of di-butyl-phthalate by soil bacteria.
445   Chemosphere, 63(8), 1377-1383.

446   Cheng, J., Liu, Y., Wan, Q., Yuan, L., Yu, X., 2018. Degradation of dibutyl phthalate in two contrasting
447   agricultural soils and its long-term effects on soil microbial community. Sci. Total Environ., 640-
448   641, 821-829.

449   Child, R., Miller, C.D., Liang, Y., Narasimham, G., Chatterton, J., Harrison, P., Sims, R.C., Britt, D.,
450   Anderson, A.J., 2007. Polycyclic aromatic hydrocarbon-degrading *Mycobacterium* isolates: their
451   association with plant roots. Appl. Microbiol. Biot., 75(3), 655-663.

452   Devriese, L.I., Witte, B.D., Vethaak, A.D., Hostens, K., Leslie, H.A., 2017. Bioaccumulation of PCBs
453   from microplastics in Norway lobster (*Nephrops norvegicus*): An experimental study. Chemosphere,
454   186, 10-16.

455   Fenner, K., Canonica, S., Wackett, L.P., Elsner, M., 2013. Evaluating pesticide degradation in the
456   environment: blind spots and emerging opportunities. Science, 341(6147), 752-758.

457   George, M.G., Don, J.B., Noel, R.K., James T.S., David R.B., Richard W.C., 2001. Bergey's Manual of
458   Systematic Bacteriology. 2nd edition. Springer Publishing Press, New York.

459   Gharasoo, M., Centler, F., Van Cappellen, P., Wick, L.Y., Thullner, M., 2015. Kinetics of substrate
460   biodegradation under the cumulative effects of bioavailability and self-inhibition. Environ. Sci.
461   Technol., 49(9), 5529-5537.

462   Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017.
463   Occurrence and effects of plastic additives on marine environments and organisms: A review.
464   Chemosphere, 182, 781-793.

465   Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L.,
466   2015. Plastic waste inputs from land into the ocean. Science, 347(6223), 768-771.

467   Johnston, A., Crombie, A.T., El, K.M., Sims, L., Whited, G.M., McGenity, T.J., Colin, M.J., 2017.
468   Identification and characterisation of isoprene-degrading bacteria in an estuarine environment.
469   Environ. Microbiol., 19(9), 3526-3537.

470   Kandil, M.M., Trigo, C., Koskinen, W.C., Sadowsky, M.J., 2015. Isolation and Characterization of a
471   novel imidacloprid-degrading *Mycobacterium* sp. strain MK6 from an Egyptian soil. J. Agr. Food
472   Chem., 63(19), 4721-4727.

473   Koelmans, A.A., Besseling, E., Foekema, E.M., 2014. Leaching of plastic additives to marine organisms.
474   Environ. Pollut., 187, 49-54.

475   Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version
476   7.0 for bigger datasets. Mol. Biol. Evol., 33(7), 1870-1874.

477   Lubecki, L., Kowalewska, G., 2019. Plastic-derived contaminants in sediments from the coastal zone of
478   the southern Baltic Sea. Mar. Pollut. Bull., 146, 255-262.

479   Lucia, P., Avio, C.G., Giuliani, M.E., Giuseppe, D., Keiter, S.H., Bettie, C., Stefania, G., Francesco, R.,
480   2018. Microplastics as vehicles of environmental pahs to marine organisms: combined chemical
481   and physical hazards to the Mediterranean mussels, *Mytilus galloprovincialis*. Front. Mar. Sci., 5,
482   103.

483   Nahurira, R., Ren, L., Song, J., Jia, Y., Wang, J., Fan, S., Wang, H., Yan, Y., 2017. Degradation of di(2-

ethylhexyl) phthalate by a novel *Gordonia alkanivorans* strain YC-RL2. Curr. Microbiol., 74(3), 309-319.

Nakamiya, K., Hashimoto, S., Ito, H., Edmonds, J.S., Yasuhara, A., Morita, M., 2005. Microbial treatment of bis (2-ethylhexyl) phthalate in polyvinyl chloride with isolated bacteria. J. Biosci. Bioeng., 99(2), 115-119.

Navacharoen, A., Vangnai, A.S., 2011. Biodegradation of diethyl phthalate by an organic-solvent-tolerant *Bacillus subtilis* strain 3C3 and effect of phthalate ester coexistence. Int. Biodeter. Biodegr., 65(6), 818-826.

Net, S., Sempéré, R., Delmont, A., Paluselli, A., Ouddane, B., 2015. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ. Sci. Technol., 49(7), 4019-4035.

Paluselli, A., Kim, S.K., 2020. Horizontal and vertical distribution of phthalates acid ester (PAEs) in seawater and sediment of East China Sea and Korean South Sea: Traces of plastic debris? Mar. Pollut. Bull., 151, 110831.

Paria, S., 2008. Surfactant-enhanced remediation of organic contaminated soil and water. Adv. Colloid. Interface. Sci., 138(1), 24-58.

Parte, A.C., 2018. LPSN - list of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. Int. J. Syst. Evol. Microbiol., 68(6), 1825-1829.

Patowary, K., Patowary, R., Kalita, M.C., Deka, S., 2017. Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. Front. Microbiol., 8, 279.

Pluskal, T., Castillo, S., Villar-Briones, A., Oresic, M., 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics, 11, 395.

Ren, L., Jia, Y., Ruth, N., Qiao, C., Wang, J., Zhao, B., Yan, Y., 2016. Biodegradation of phthalic acid esters by a newly isolated *Mycobacterium* sp. YC-RL4 and the bioprocess with environmental samples. Environ. Sci. Pollut. R., 23(16), 16609-16619.

Ren, L., Lin, Z., Liu, H., Hu, H., 2018. Bacteria-mediated phthalic acid esters degradation and related molecular mechanisms. Appl. Microbiol. Biot., 102(3), 1085-1096.

Savoca, D., Arculeo, M., Barreca, S., Buscemi, S., Caracappa, S., Gentile, A., Persichetti, M.F., Pace, A., 2018. Chasing phthalates in tissues of marine turtles from the Mediterranean sea. Mar. Pollut. Bull., 127, 165-169.

Schiedek, T., 1995. Impact of plasticizers (phthalic acid esters) on soil and groundwater quality. Groundwater Quality: Remediation and Protection, IAHS Press, Wallingford, 149-156.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ. Sci. Technol., 38(12), 228-231.

Sutherland, T.D., Horne, I, Harcourt, R.L., Russell, R.J., Oakeshott, J.G., 2002. Isolation and characterization of a *Mycobacterium* strain that metabolizes the insecticide endosulfan. J. Appl. Microbiol., 93(3), 380-389.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A., McGonigle, D., Russell, A.E., 2004. Lost at sea: Where is all the plastic? Science, 304(5672), 838-838.

Wang, Y., Zhan, W., Ren, Q., Cheng, S., Wang, J., Ma, X., Zhang, C., Wang, Y., 2019. Biodegradation of di-(2-ethylhexyl) phthalate by a newly isolated *Gordonia* sp. and its application in the

528   remediation of contaminated soils. Sci. Total Environ., 689, 645-651.

529   Wright, R.J., Bosch, R., Gibson, M.I., Christie-Oleza, J.A., 2020. Plasticizer degradation by marine
530   bacterial isolates: a proteogenomic and metabolomic characterization. Environ. Sci. Technol., 54(4),
531   2244-2256.

532   Wu, Y., Xu, M., Xue, J., Shi, K., Gu, M., 2019. Characterization and enhanced degradation potentials of
533   biosurfactant-producing bacteria isolated from a marine environment. ACS omega, 4(1), 1645-1651.

534   Xu, Y., Liu, X., Zhao, J., Huang, H., Wu, M., Li, X., Li, W., Sun, X., Sun, B., 2021. An efficient phthalate
535   ester-degrading *Bacillus subtilis*: Degradation kinetics, metabolic pathway, and catalytic
536   mechanism of the key enzyme. Environ. pollut., 273, 116461-116461.

537   Yamada, H., Yamaguchi, M., Igarashi, Y., Chikamatsu, K., Aono, A., Murase, Y., Morishige, Y., Takaki,
538   A., Chibana, H., Mitarai, S., 2018. *Mycolicibacterium smegmatis*, basonym *Mycobacterium*
539   *smegmatis*, expresses morphological phenotypes much more similar to *Escherichia coli* than
540   *Mycobacterium tuberculosis* in quantitative structome analysis and CryoTEM examination. Front.
541   Microbiol., 9, 1992.

542   Yang, T., Ren, L., Jia, Y., Fan, S., Wang, J., Wang, J., Nahurira, R., Wang, H., Yan, Y., 2018.
543   Biodegradation of di-(2-ethylhexyl) phthalate by *Rhodococcus ruber* YC-YT1 in contaminated
544   water and soil. Int. J. Env. Res. Pub. He., 15(5), 964.

545   Yu, H., Wang, L., Lin, Y., Liu, W., Tuyiringire, D., Jiao, Y., Zhang, L., Meng, Q., Zhang, Y., 2020.
546   Complete metabolic study by dibutyl phthalate degrading *Pseudomonas* sp. DNB-S1. Ecotox.
547   Environ. Safe., 194, 110378.

548   Zhang, H., Lin, Z., Liu, B., Wang, G., Weng, L., Zhou, J., Hu, H., He, H., Huang, Y., Chen, J., Ruth, N.,
549   Li, C., Ren, L., 2020. Bioremediation of di-(2-ethylhexyl) phthalate contaminated red soil by
550   *Gordonia terrae* RL-JC02: Characterization, metabolic pathway and kinetics. Sci. Total Environ.,
551   733, 139138.

552   Zhang, J., Zhao, R., Cao, L., Lei, Y., Liu, J., Feng, J., Fu, W., Li, X., Li, B., 2020. High-efficiency
553   biodegradation of chloramphenicol by enriched bacterial consortia: Kinetics study and bacterial
554   community characterization. J. Hazard. Mater., 384, 121344.

555   Zhang, Q., Song, J., Li, X., Peng, Q., Yuan, H., Li, N., Duan, L., Ma, J., 2019. Concentrations and
556   distribution of phthalate esters in the seamount area of the Tropical Western Pacific Ocean. Mar.
557   Pollut. Bull., 140, 107-115.

558   Zhao, H., Hu, R., Chen, X., Chen, X., Lü, H., Li, Y., Li, H., Mo, C., Cai, Q., Wong, M., 2018.
559   Biodegradation pathway of di-(2-ethylhexyl) phthalate by a novel *Rhodococcus pyridinivorans* XB
560   and its bioaugmentation for remediation of DEHP contaminated soil. Sci. Total Environ., 640-641,
561   1121-1131.

562   Zhao, H.M., Du H, Huang, C.Q., Li, S., Zeng, X.H., Huang, X.J., Xiang, L., Li, H., Li, Y.W., Cai, Q.Y.,
563   Mo, C.H., He, Z., 2019. Bioaugmentation of exogenous strain *Rhodococcus* sp. 2G can efficiently
564   mitigate di(2-ethylhexyl) phthalate contamination to vegetable cultivation. J. Agric. Food Chem.,
565   67(25), 6940-6949.