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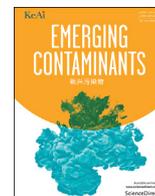
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# Insights into bacterial resistance to contaminants of emerging concerns and their biodegradation by marine bacteria



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## ABSTRACT

Contaminants of Emerging Concern (CECs) are human-made chemicals that remain unregulated. The continuous detection of CECs in aquatic ecosystems, due to their incomplete removal, emphasizes the importance of understanding their fate and impact on the environment and human health. The detrimental effects of CECs on marine eukaryotes are well documented in multiple studies. However, their impact on marine bacteria and their biodegradation by these organisms are not well understood. In this study, two marine bacteria, *Priestia* sp. 35 ODPABA G14 and *Rhodococcus* sp. 23 AHTN G14, previously isolated from submarine sediments, were used. These two strains were tested for their resistance as well as their capacity to degrade different classes of hydrophobic and hydrophilic CECs, including synthetic musks, UV filters, pesticides and pharmaceuticals. Both strains showed high resistance to all of the hydrophobic tested CECs even up to 500 mg L<sup>-1</sup>. Only Ketoprofen was toxic to bacterial cells, particularly to *Rhodococcus* sp. starting at concentration as low as 4 mg L<sup>-1</sup>. Furthermore, *Priestia* sp. and *Rhodococcus* sp. strains exhibited high biodegradation potential, especially for hydrophobic compounds. Although this may not apply to all pollutants, the data presented in this study suggest a positive correlation between marine bacterial resistance to CECs and their high biodegradation potentials.

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## 1. Introduction

Over the past 20 years, the term ‘emerging’ has been used in numerous studies focusing on non-regulated substances. Contaminants of Emerging Concern (CECs) are synthetic organic compounds, including personal care products, pharmaceuticals, sanitation products, industrial chemicals and pesticides that are not currently regulated or widely monitored [1]. Numerous CECs have been detected in environmental compartments, such as surface waters, groundwaters, and wastewaters [1,2], as well as sediments [3], and have been accumulated in marine organisms [4]. Pharmaceuticals and personal care products are among the most frequently detected CECs in environmental water samples [5,6], however several CECs still lack regulation. The main issue with

CECs is the increase of their concentration in a number of environmental compartments and the lack of knowledge regarding their long-term impact on the aquatic environment and human health.

Various studies have investigated the ecotoxicological risks posed by CECs on marine eukaryotes [7]. For instance, UV filters are known to impact the hormonal system of fishes [8,9]. In addition, two UV filters, 2-Ethyl-hexyl-4-methoxycinnamate (EHMC) and 4-Methylbenzylidene-camphor (4-MBC) are identified as the most toxic for the marine organisms *Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Siriella armata*, with toxicity threshold ranging from µg L<sup>-1</sup> to mg L<sup>-1</sup> [10]. The acute toxicity of musks such as galaxolide (HHCB) and tonalide (AHTN) to various aquatic organisms including *Nitocra spinipes* [11], *Acartia Tonsa* [12], *Lampsilis cardium* [13], *Oryzias latipes* [14], *Scenedesmus quadricauda* and *Navicula* sp. [15] was evaluated with their LC<sub>50</sub> ranging from 0.02 to 12 mg L<sup>-1</sup>. Additionally, *Danio rerio* [16] and *Pseudokirchneriella subcapitata* [17] exhibited lowest observed effect

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**Abbreviations**

CEC(s)	contaminant(s) of emerging concern
EHMC	hexyl-4-methoxycinnamate
4-MBC	4-Methylbenzylidene-camphor
HHCB	galaxolide
AHTN	tonalide
LOEC	lowest observed effect concentration
NOEC	no observed effect concentration
WWTP(s)	wastewater treatment plant(s)
OC	octocrylene
BP3	benzophenone-3/2-Hydroxy-4-methoxybenzophenone

NSAID(s)	non-steroidal anti-inflammatory drug(s)
OD PABA	padimate-O
KTP	ketoprofen
OXA	oxazepam
ATZ	atrazine
MX-d15	musk xylene-d15
EtoAc	ethyl acetate
2HB	2-Hydroxybenzophenone
BP4	2-Hydroxy-4 methoxybenzophenone-5-sulfonicacid
DBP	di-n-butyl phthalate
CSH	cell surface hydrophobicity

concentration (LOEC) or no observed effect concentration (NOEC) at concentrations as high as  $0.4 \text{ mg L}^{-1}$ . Pharmaceuticals, such as ibuprofen, ketoprofen, novobiocin and carbamazepine, are also toxic and affect the behavioral and physiological parameters of the Mollusca *Ruditapes philippinarum* [18], the Annelida *Hediste diversicolor* [19], and the Arthropoda *Daphnia magna* [20], with concentrations as low as  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$ .

Despite the large availability of data on the ecotoxicological risks of CECs on marine eukaryotes, their effects on marine microbial communities, particularly marine bacteria, have not been studied closely. Few studies have demonstrated that pharmaceuticals and UV filters are detrimental to the growth of the marine bacteria *Aliivibrio fischeri* [21] and *Photobacterium leiognathi* [22] with toxic concentrations ranging from  $1$  to  $9 \text{ mg L}^{-1}$ , similar to the most resistant marine eukaryotes. However, other studies have shown that *A. fischeri* [23], *Bacillus thuringiensis* B1 [24] and *Alkanotrophic rhodococci* [25] are resistant to CECs at concentrations greater than  $250 \text{ mg L}^{-1}$ . The difference in resistance capacity among bacterial strains to CECs could be attributed to the chemical properties of CECs (e.g., hydrophobicity/hydrophilicity), as well as to the ability of bacterial strains to transform or degrade various organic compounds, including their use as sources of carbon and energy [26,27].

Microbial mineralization and/or degradation (for simplicity the term biodegradation will be used) is one of the key processes governing the transport and fate of organic pollutants in the environment. A wide array of microorganisms, including fungi, protozoa, bacteria, etc., can accomplish these processes [28]. Many studies have investigated the microbial degradation of various organic pollutants, including CECs, because of their potential application in bioremediation [29]. These studies have been extensively conducted in a range of conditions from pure isolated cultures to complex environments such as sediments and wastewater treatments sludges, in order to understand the mechanisms of organic pollutant biodegradation. For instance, natural microbial communities in marine sediments have been reported to remove various CECs [30,31]. In engineered systems like wastewater treatment plants (WWTPs), the biodegradation of CECs have been achieved by exploiting the catabolic activities of microbial consortia in activated sludge [32,33]. However, WWTP sludge may exhibit varying degradation capacities for different CECs, with some being degraded effectively, whilst others show limited or no degradation [34].

Multiple studies have focused on the isolation and identification of bacteria which can degrade organic pollutants from the environment, however finding a pure bacterial strain that can optimally degrade one or multiple selected compounds is a challenge. Bacterial strains from the Genera *Bacillus/Priestia*, *Pseudomonas*,

*Sphingomonas*, *Flavobacterium*, *Nocardia*, *Rhodococcus*, and *Mycobacterium* can degrade a variety of organic compounds including pesticides, alkanes, hydrocarbons, or polyaromatic compounds [35]. To name a few, the removal of octocrylene (OC) was determined by *Mycobacterium agri* with biofilm formation isolated from a landfill site, *Gordonia* sp. strain OC\_S5, and *Sphingopyxis* sp. strain OC\_4D isolated from WWTP sludge [34,36]. *Sphingomonas wittichii* strain BP14P isolated from WWTP also degrades UV filter benzophenone-3 (BP3) [37] effectively. Among non-steroidal anti-inflammatory drugs (NSAIDs), *Bacillus thuringiensis* removed ibuprofen and naproxen [38] more efficiently and diclofenac was rapidly degraded by *Rhodococcus ruber* IEGM 346 [39].

Coastal and marine ecosystems are crucial endpoints and accumulation areas of CECs originating from both point and non-point sources of contamination [40]. In a previous study by Azaroff et al. (2020), high concentrations of CECs such as musks (HHCB and AHTN), UV filters (OC and padimate-O (OD PABA)) and pharmaceuticals were detected in the submarine Capbreton canyon (Southwest of France) sediments ranging from  $0.8$  to  $29.2 \text{ mg kg}^{-1}$ . This study illustrated the transfer and accumulation of CECs along the canyon [3]. Sediment slurry incubations from the same sampling area demonstrated biotic degradation potential for HHCB, OD PABA, and carbamazepine [31]. Bacterial strains were isolated from these marine sediments, enriched with a single CEC, and examined for their ability to degrade the CEC used for enrichment. The preliminary findings of this study demonstrated that bacteria isolated from marine sediments of the Capbreton canyon mainly belong to the *Actinomycetota* and *Bacillota* phyla (mainly *Bacillus/Priestia* and *Rhodococcus* genera) and may have significant degradation potential for CECs [31].

The isolated bacteria from the Capbreton canyon serve as excellent models for studying the interplay between both the toxic effect of CECs on marine bacteria and their potential for biodegradation of CECs. In this study, *Priestia* sp. 35 OD PABA G14 (strain S-1, previously named *Bacillus* sp. 35 OD PABA G14) and *Rhodococcus* sp. 23 AHTN G14 (strain S-2) (Table 1) isolated from Capbreton canyon sediments and capable of degrading, OD PABA and AHTN, respectively, were assessed for their resistance to various CECs including synthetic musks (galaxolide, HHCB; tonalide, AHTN), UV filters (Octocrylene, OC; Padimate-O, OD PABA), and pharmaceuticals (Ketoprofen, KTP). These strains along with other isolated *Bacillus* sp. strains (strains S-3 and S-4) from the same canyon, were tested for their ability to degrade selected CECs by adding another pharmaceutical (Oxazepam, OXA) and a pesticide (Atrazine, ATZ), taking into account the hydrophobic and hydrophilic characteristics of the CECs, along with toxicity tests.

## 2. Material and methods

### 2.1. CECs standard solutions

Different compounds were used for the experiment: HHCB from TRC (95% purity; (Toronto, Canada)), AHTN from LGC (Molsheim, France), OC, OD PABA, and ATZ from Sigma-Aldrich (Saint-Louis, USA). Other chemical compounds KTP, OXA, classified as analytical grade (>98%), were also obtained from Sigma-Aldrich. The internal standards were Carbamazepine-d10, Nordiazepam-d5, Atrazine-d5 from Sigma-Aldrich and Musk Xylene-d15 from LGC (MX-d15, 96.8% purity, 100 ng  $\mu\text{L}^{-1}$  in acetone). HHCB and ATZ were prepared in acetonitrile, AHTN, OD PABA and OC were prepared in 2-propanol, KTP and OXA were prepared in methanol, and were all stored at  $-20\text{ }^{\circ}\text{C}$ .

Table S1 summarizes the main characteristics of the emerging contaminants used in this study namely hydrophobic (HHCB, AHTN, OC, OD PABA; log  $K_{\text{OW}}$  between 5 and 7) and hydrophilic (KTP, OXA, ATZ; log  $K_{\text{OW}}$  between 2 and 4) compounds.

### 2.2. Bacterial strains and culture

The bacterial strains (S-1, S-2, S-3, and S-4) were previously isolated from canyon sediments [31], contaminated with many pollutants, including CECs. Each strain was isolated on a medium enriched (MM<sub>20</sub>) with a single CEC that included either AHTN, OC, or OD PABA. Table 1 summarizes the isolated strains and the compounds used for their isolation, and their respective phyla and genera. Only S-1, S-2, and S-4 have been examined for their biodegradation capacity of the specific pollutant used for their isolation in previous work [31]. These strains were selected on the basis of their high degrading potential, as indicated in the previous work. Strain S-2 was selected because it belongs to a different genus than S-1, S-3 and S-4. S-3 which was partially isolated in previous study [31], was re-plated on Luria-Bertani solid medium to obtain pure strains in the present study.

For all experiments, isolated colonies were cultured to obtain a pre-culture prior to each experiment. The strains were cultured in MM<sub>5</sub> medium (MM<sub>5</sub> is MM<sub>20</sub> [31] prepared with 5 g  $\text{L}^{-1}$  NaCl instead of 5 g  $\text{L}^{-1}$  NaCl) supplemented with 2.5 g yeast extract, 5 g peptone, and 5 g glucose. The cells were cultured aerobically at  $30\text{ }^{\circ}\text{C}$ . To monitor cell growth, the optical density (OD) was monitored at 600 nm at the appropriate times during the experiments. After growth, the cells were centrifuged at 6000 rpm for 5 min, and pellets were re-suspended in fresh medium before inoculation for toxicity tests as well as CEC degradation tests.

### 2.3. Toxicity tests

The CEC toxicity tests were conducted to determine the influence of CECs on the growth of bacterial strains. CECs were individually plated in 96-well microplates (Evergreen) at concentrations ranging from 0 to 500 mg  $\text{L}^{-1}$ . The CEC-containing solvents were evaporated in each well before bacterial inoculation. The freshly inoculated MM<sub>5</sub> medium with pre-cultures of

strains S-1 and S-2 were introduced into each well of the microplate to a final volume of 200  $\mu\text{L}$ . The microplates were incubated at  $30\text{ }^{\circ}\text{C}$  and shaken at 200 rpm, growth was monitored at 600 nm using a SYNERGY HTX multimode reader (BioTek) every hour until the stationary phase. The  $\Delta\text{OD}$ , monitored at 600 nm, was calculated as  $\text{OD}_{\text{max}} - \text{OD}_{\text{min}}$  and the growth rate ( $\mu$ ) was determined using the formula:  $\mu = \text{Ln}(N_t/N_0)/t$ , where  $N_t$  is the cell count at exponential phase,  $N_0$  is the initial cell count, and  $t$  is the incubation time at  $N_t$ .

### 2.4. Experimental CECs degradation test

The degradation test protocol has been previously described in Ref [31]. Briefly, degradation tests were conducted in sterile glass tubes containing evaporated contaminants (HHCB, AHTN, OC, OD PABA, KTP, OXA, and ATZ) at a final concentration of 1 mg  $\text{L}^{-1}$  in triplicate, along with abiotic controls (without bacterial strains) for each CEC. Pre-cultured cells were used to inoculate fresh medium containing contaminants. Sampling was conducted at the initial time ( $T_0$ ) and final time ( $T_f$ ) after 24 h for all strains of *Priestia* sp./*Bacillus* sp. and 48 h for *Rhodococcus* sp.. Incubation was stopped by adding cells directly into vials containing ethyl acetate (EtoAc) at a 1:1 ratio for HHCB, AHTN, OC, OD PABA and ATZ (GC-MS). For KTP and OXA, the cultures were frozen with liquid nitrogen (for LC-MS/MS analysis) to stop incubation (see 2.5 CECs analysis). All the glass tubes and vials used in this experiment were washed twice with acetone overnight and subjected to pyrolysis.

### 2.5. CECs analysis

#### 2.5.1. Sample preparation previous analysis

A 1 mL culture incubated with either HHCB, AHTN, OC, OD PABA, or ATZ was extracted with 1 mL ethyl acetate (EtOAc). Following manual shaking for 5 min, the tubes were then centrifuged to facilitate the separation of the organic phase. KTP and OXA were diluted by a factor 2 in MeOH/water (25/75 v/v) and 0.1% of formic acid spiked with internal standards (Carbamazepine-d10 and Nordiazepam-d5). The diluted samples were filtered through a 0.2  $\mu\text{m}$  polytetrafluoroethylene (PTFE) filter and transferred into vials. Finally, the samples were stored at  $-20\text{ }^{\circ}\text{C}$  until analysis.

#### 2.5.2. GC – MS measurements

HHCB, AHTN, OC, OD PABA, and ATZ were analyzed by GC/MS (Agilent Technologies, 7890A, inert MSD (mass selective detector with triple-axis detector). The method was adapted from Miossec et al. [41]. The system was equipped with the single taper ultra-inert liner with glass wool and a HP-5MS UI capillary column (30 m length x 250  $\mu\text{m}$  diameter x 0.25  $\mu\text{m}$  film thickness) with the carried gas helium at greater than 99.999 % purity (Linde). A six-point calibration curve was performed in EtOAc spiked with standards ranging from 0 to 666  $\mu\text{g L}^{-1}$ , as well as internal standard at 150  $\mu\text{g L}^{-1}$ .

#### 2.5.3. LC – MS/MS measurements

The concentration of KTP and OXA were determined by LC-MS/

**Table 1**  
Pure bacterial strains used in this study.

Code	Bacterial strains	Compound used for isolation	Phylum	Genus
S-1	35 OD PABA G14	OD PABA	<i>Bacillota (Firmicutes)</i>	<i>Priestia</i>
S-2	23 AHTN G14	AHTN	<i>Actinomycetota (Actinobacteria)</i>	<i>Rhodococcus</i>
S-3	AHTN G14	AHTN	<i>Bacillota (Firmicutes)</i>	<i>Bacillus</i>
S-4	40 OC G14	OC	<i>Bacillota (Firmicutes)</i>	<i>Bacillus</i>

MS (Waters, Acquity UPLC H-Class). The method was adapted from Miossec et al. [42]. The system was equipped with an Acquity UPLC HSS T3 column (1.8  $\mu\text{m}$  particle size, 2.1 mm  $\times$  50 mm (Waters)) preceded by a guard column (1.8  $\mu\text{m}$  particle size, 2.1  $\times$  5 mm) of the same packing material. The flow rate of the mobile phase was 0.4 mL  $\text{min}^{-1}$ . Quantification was obtained within a six-point calibration curve ranging from 0 to 800  $\mu\text{g L}^{-1}$  of standards and 53  $\mu\text{g L}^{-1}$  of the internal standard in MeOH/Water (25/75 v/v) and 0.1% formic acid.

## 2.6. Calculation of CEC degradation by bacterial strains

The degradation percentages of CECs were calculated by subtracting the CECs concentration at the final time ( $\text{CEC}_{T_f}$ ) from the mean concentration at the initial time ( $\text{CEC}_{T_0, \text{mean}}$ ), dividing this by the mean concentration at the initial time ( $\text{CEC}_{T_0, \text{mean}}$ ) for each replicate using the following equation:

$$\text{degradation of CEC (\%)} = \frac{[\text{CEC}_{T_0, \text{mean}} - \text{CEC}_{T_f}]}{[\text{CEC}_{T_0, \text{mean}}]} * 100$$

The mean of three replicates was determined along with the standard deviation.

## 2.7. Statistical analysis

The experimental data were checked for assumptions of homogeneity of variance across treatments using the Shapiro-Wilk test. When the assumptions were met, significant differences in bacterial growth with concentrations and degradation ratios between the compounds and controls were analyzed by one-way analysis of variance (ANOVA), followed by pairwise comparisons of means. Additionally, a multiway ANOVA was applied to analyze the growth of bacteria with concentrations and pollutant factors. When homogeneity was not observed in the data, the nonparametric Kruskal–Wallis test was used. Differences were considered significant at  $p < 0.05$ . All statistical analyses were performed using R-Studio.

## 3. Results and discussion

### 3.1. Evaluation of CEC toxicity on S-1 and S-2 strains

To assess the toxicity of CECs on marine bacterial strains from *Bacillota* and *Actinomycetota* phyla, we selected the *Priestia* sp. 35 OD PABA G14 (S-1) and *Rhodococcus* sp. 23 AHTN G14 (S-2) strains, known for their ability to degrade OD PABA and AHTN respectively [43]. The toxicity tests covered a concentration range of 0–500  $\text{mg L}^{-1}$  (tested at concentrations from 2 to 60  $\text{mg L}^{-1}$  and then from 100 to 500  $\text{mg L}^{-1}$ ) to determine the toxicity threshold of the compounds across different classes of CECs, including synthetic musks (HHCB and AHTN), UV filters (OC and OD PABA) and pharmaceutical (KTP). Fig. 1 shows the  $\Delta\text{OD}$  ( $\text{OD}_{\text{max}} - \text{OD}_{\text{min}}$ ), representing production as well as the calculated exponential growth rate ( $\mu$  expo).

#### 3.1.1. S-1 and S-2 strains resist HHCB, AHTN, OC and OD PABA up to 500 $\text{mg L}^{-1}$

In the absence of pollutants, the calculated growth rates were up to 0.45 and 0.09  $\text{h}^{-1}$  for S-1 and S-2, respectively (Fig. 1). No significant differences were observed in the growth of both species in the presence of HHCB, AHTN, or OC, even up to 500  $\text{mg L}^{-1}$  ( $p$ -value  $> 0.05$ ) (Fig. 1). While OD PABA did not significantly affect the growth of strain S-1 (Fig. 1) ( $p$ -value  $> 0.05$ ), a noticeable effect on

the growth of S-2 was recorded after 4  $\text{mg L}^{-1}$  (Fig. 1) ( $p$ -value  $< 0.05$ ). Although a decrease in the growth rate of S-2 was observed up to 60  $\text{mg L}^{-1}$  for OD PABA, complete growth inhibition was not recorded, even up to 500  $\text{mg L}^{-1}$ .

Therefore, the S-1 (*Priestia* sp. 35 OD PABA G14) and S-2 (*Rhodococcus* sp. 23 AHTN G14) strains of *Bacillota* and *Actinomycetota* phyla, can be considered resistant to synthetic musks (HHCB, AHTN) and UV-filter (OC) at concentrations up to 500  $\text{mg L}^{-1}$  of CECs. These findings align with a study on the toxicity of various UV filters on diverse marine bacteria, including *Actinomycetota*, *Bacillota*, *Bacteroidota* and *Proteobacteria* [44]. In that study, 20 out of the 27 bacterial species tested exhibited resistance to UV filters. Similarly, the Gram-negative *Stenotrophomonas maltophilia*, isolated from the drinking water distribution system, showed resistance to HHCB and AHTN [45]. It is worth noting that lower concentrations, in order of 100 and 1000 magnitudes, were tested in both previous studies compared to the current investigation. Further exposure of these bacteria [44,45] to higher CEC concentrations is necessary for an effective comparison.

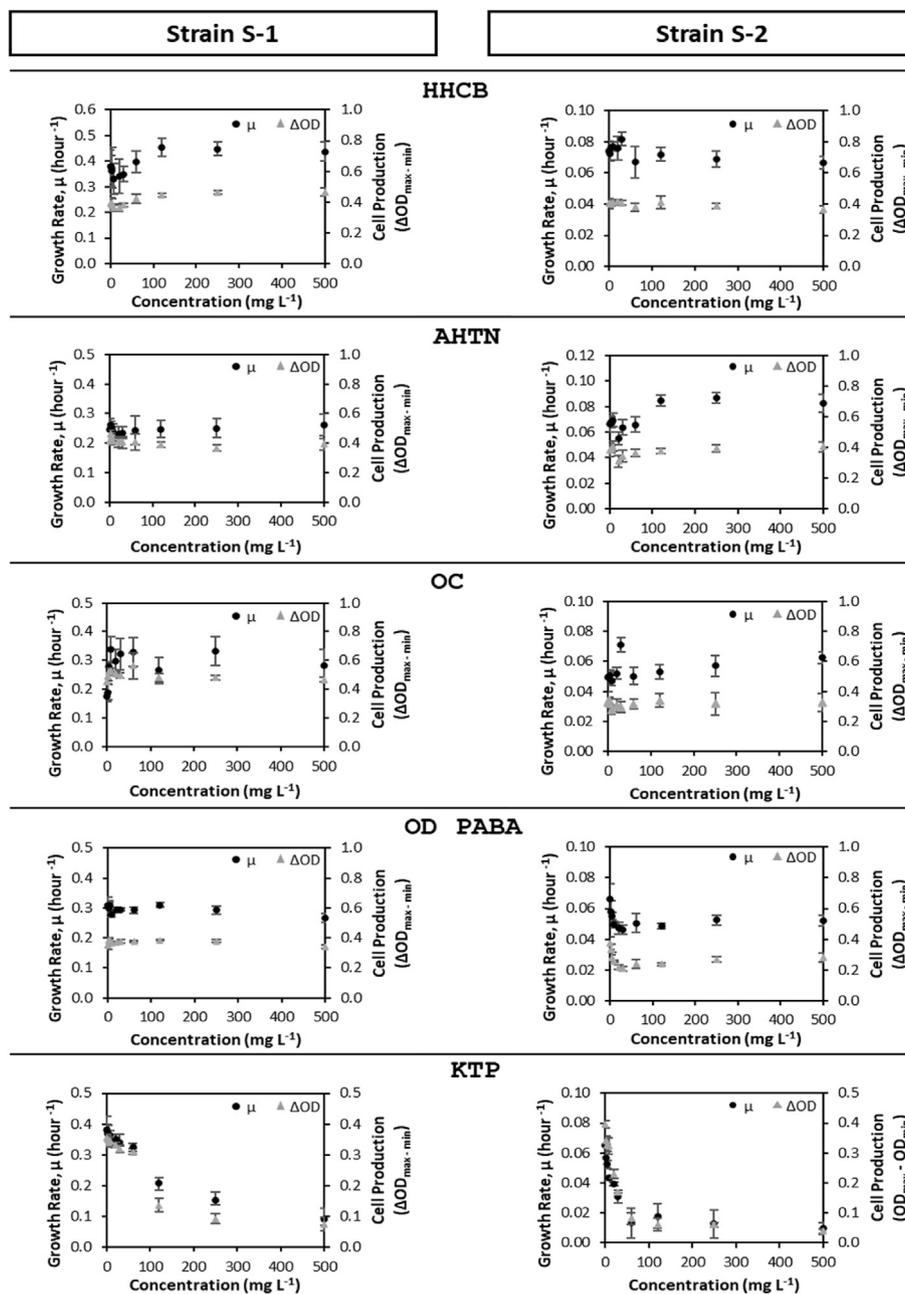
In some cases, bacterial resistance to CECs appears to be strain- and CEC-dependent. For example, the growth of the Gammaproteobacterium *Aliivibrio fischeri* is inhibited by UV filters such as zinc oxide nanoparticles and 2-ethylhexyl-4-methoxycinnamate (EHMC) with  $\text{EC}_{50}$  values as low as 8.57 and 1.06  $\text{mg L}^{-1}$ , respectively [21]. Similarly, as benzophenone (BP), 2-Hydroxybenzophenone (2HB), and BP3, inhibit *Aliivibrio fischeri* growth with  $\text{EC}_{50}$  values ranging from 13 to 18  $\text{mg L}^{-1}$  [23]. However, *Aliivibrio fischeri* shows resistance to 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid (BP4) with an  $\text{EC}_{50}$  as high as 858.95  $\text{mg L}^{-1}$  [23]. This pattern is also observed for OD PABA in our study. While OD PABA has no effect on strain S-1 (*Priestia* sp.), this UV filter partially inhibits the growth of S-2 (*Rhodococcus* sp.) up to 60  $\text{mg L}^{-1}$ , with no complete inhibition seen even up to 500  $\text{mg L}^{-1}$ . Currently, there are no studies on the effects of OD PABA on marine bacteria to explain the observed differences in its effect on strains S-1 and S-2. However, OD PABA is less hydrophobic than HHCB, AHTN, and OC (Table S1). It is plausible that the OD PABA-induced toxicity in strain S-2 is associated with the availability of OD PABA for the S-2 bacterial culture, causing partial toxicity up to 60  $\text{mg L}^{-1}$ . Beyond this concentration, OD PABA may become less available to the S-2 strain, minimizing its toxicity.

#### 3.1.2. Strains S-1 and S-2 are sensitive to KTP

In contrast to the other CECs, the addition of KTP induced toxicity in both strains S-1 and S-2 (Fig. 1). The growth of strain S-1 was inhibited at concentrations starting from 30  $\text{mg L}^{-1}$ , while the growth of strain S-2 was inhibited starting from 2  $\text{mg L}^{-1}$ , reaching complete inhibition at 30  $\text{mg L}^{-1}$ . Significant inhibition values were observed starting at 30  $\text{mg L}^{-1}$  for strain S-1 and 4  $\text{mg L}^{-1}$  for strain S-2 ( $p < 0.05$ ).

The toxicity of KTP on marine bacteria has not been well documented, with only a few studies reporting the effects of other NSAIDs on bacterial species [22,24,25]. For instance, the luminescent Gammaproteobacterium *Photobacterium leiognathi* exhibited an  $\text{EC}_{50}$  ranging from 1 to 5  $\text{mg L}^{-1}$  in the presence of various NSAIDs [22]. However, other bacteria have shown resistance to NSAIDs. For instance, *Bacillus thuringiensis* B1 sp. demonstrated resistance to ibuprofen up to 809.3  $\text{mg L}^{-1}$  [24] and *Rhodococcus* strains have previously been identified as resistant to paracetamol and acetylsalicylic acid, with minimum bactericidal concentrations ranging from 250  $\text{mg mL}^{-1}$  to 500  $\text{mg mL}^{-1}$  [25].

These findings highlight resistance to the tested CECs in this study of marine bacteria S-1 and S-2, of the *Priestia* and *Rhodococcus* genera isolated from the Capbreton canyon sediments. Notably, this resistance is particularly evident for CECs with a



**Fig. 1.** The growth rate ( $\mu$  expo) and cell production ( $OD_{max} - OD_{min}$ ) measured at 600 nm for all CECs (synthetic musks (HHCB and AHTN), UV filters (OC and OD PABA) and pharmaceutical (KTP) by S-1 (left side) and S-2 (right side) at different concentrations.

higher hydrophobicity index than for marine eukaryotes. However, it is essential to note that these observations may not necessarily extend to other bacterial strains isolated from different environmental compartments, because the toxic effects of CECs appear to be both strain-dependent and compound-dependent.

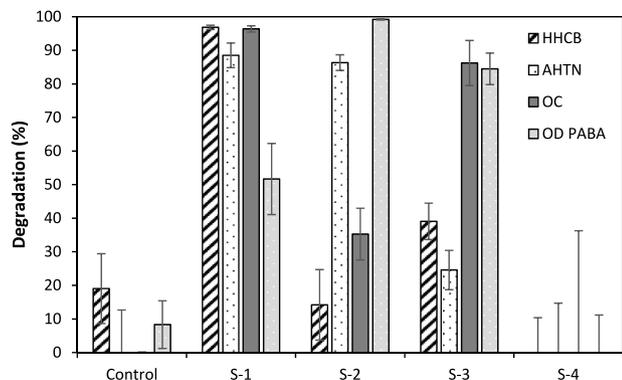
### 3.2. Degradation of CECs by *Bacillota* and *Actinomycetota*

To establish a correlation between the resistance of marine bacterial strains S-1 and S-2 to hydrophobic CECs and their potential for biodegradation, biodegradation tests were conducted with the two strains using both hydrophobic (HHCB, AHTN, OC, and OD PABA) and hydrophilic (KTP along with OXA and ATZ) CECs. To broaden our understanding of CEC biodegradation capacity in the submarine Capbreton canyon, we included additional pure

bacterial strains from the *Bacillus* genus, namely S-3 and S-4 (Table 1).

#### 3.2.1. Degradation potential of hydrophobic compounds (HHCB, AHTN, OC, and OD PABA)

The degradation tests using hydrophobic compounds HHCB, AHTN, OC, and OD PABA as substrates are summarized in Fig. 2. As expected, no significant degradation was recorded in the abiotic control for all the treatments ( $p$ -value  $0.346 > 0.05$ ). Notably, S-1 ( $p$ -value  $< 0.001$ ), S-2 ( $p$ -value  $0.018 < 0.05$ ), and S-3 ( $p$ -value  $0.003 < 0.05$ ) exhibited robust degradation abilities for hydrophobic compounds. Indeed, S-1 was the most efficient in degrading hydrophobic compounds, with a depletion range of  $89 \pm 4\%$  to  $97 \pm 1\%$  for HHCB, AHTN, and OC, and  $52 \pm 11\%$  for OD PABA. In comparison, S-2 demonstrated degradation percentages of  $14 \pm 11\%$



**Fig. 2.** Degradation percentage of hydrophobic compounds (musk; HHCB and AHTN, UV filters; OC and OD PABA) using selected isolated strains (Table 1). Abiotic control (control) was performed without inoculum in medium MM<sub>20</sub>. Initial exposure concentration was 1 mg L<sup>-1</sup>. Data are mean ± SD of three replicates.

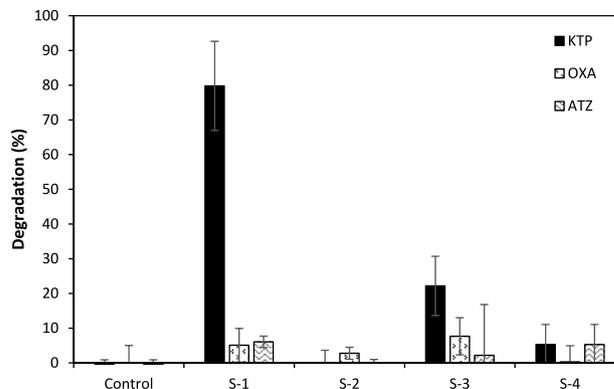
for HHCB, 86 ± 2% for AHTN, 35 ± 8% for OC, and complete degradation (99 ± 0%) of OD PABA. Similarly, S-3 showed degradation rates ranging from 25 ± 6% to 86 ± 7% for HHCB, AHTN, OC, and OD PABA. Conversely, S-4 showed no potential for the removal of hydrophobic compounds.

### 3.2.2. Degradation potentials of hydrophilic compounds (KTP, OXA and ATZ)

The pharmaceutical hydrophilic compounds KTP and OXA were used as the substrates for degradation tests (Fig. 3). Additionally, the biodegradation of the hydrophilic compound ATZ, known to impact the growth of *Bacillus* sp. at concentrations as low as 0.5 mg L<sup>-1</sup> [46] was tested. Like the results with hydrophobic compounds, no significant loss was observed in the degradation experiments with the abiotic control ( $p$ -value 0.629 > 0.05). ATZ and OXA were not significantly degraded by any of the strains tested. However, while S-2 and S-4 showed no ability to degrade KTP, 80 ± 13 and 22 ± 9% degradation were recorded for S-1 and S-3, respectively.

The results presented in Figs. 2 and 3 show that the S-1, S-2 and S-3 strains belonging respectively to *Priestia*, *Rhodococcus*, and *Bacillus* genera can each degrade various classes of CEC compounds. Different *Priestia* (previously *Bacillus*) and *Bacillus* genera strains are known for their ability to biodegrade various classes of organic pollutants. However, few studies have shown that strains of *Bacillus*/*Priestia* and *Rhodococcus* genera can each degrade different classes of organic pollutants. For example, *Bacillus thuringiensis* isolated from polluted marine sediments exhibits the potential to degrade phenanthrene and imidacloprid up to 80% [47], whereas *Bacillus cereus* isolated from petroleum sludge demonstrated the capability to degrade various aromatic and emerging pollutants [48]. Similarly, few strains of *Rhodococcus* can degrade a number of different organic pollutants. For instance, *Rhodococcus aetherivorans* can degrade phenols [49] and hydrocarbons [50,51].

The noteworthy discovery in this study was the differential biodegradation potential shown by two distinct bacterial strains of the *Bacillus* genus, namely S-3 and S-4. Despite both strains being isolated from sediment samples contaminated with a number of different CECs [31], only strain S-3 displayed a high biodegradation potential across multiple classes of CECs. Biodegradation necessitates the adsorption and transport of chemical compounds into the cell, as well as the production of specific cellular enzymes as biocatalysts. Various cellular pathways have been identified for the biodegradation of organic pollutants [52]. To name a few, monooxygenases and dioxygenases are involved in the process of



**Fig. 3.** Degradation percentage of hydrophilic compounds (Pharmaceuticals; KTP and OXA, Pesticide; ATZ) using selected isolated strains (Table 1). Abiotic control (control) was performed without inoculum in medium MM<sub>20</sub>. Initial exposure concentration was 1 mg L<sup>-1</sup>. Data are mean ± SD of three replicates.

desulfurization, dehalogenation, and hydroxylation of aromatic and aliphatic compounds. The monooxygenase cytochrome P450, for instance, is well-documented in the biodegradation of various organic pollutants [53–55]. Laccases and peroxidases contribute to the oxidation of a variety of organic pollutants, such as those of emerging concern [56]. Future studies should explore the presence of these metabolic pathways in different bacterial species, assess their roles in biodegradation, and investigate their contributions to the removal efficiencies of one or multiple compounds.

It is crucial to highlight the diverse chemical properties of the selected CECs, including factors such as molecular weight, atomic structure, and the number and positions of aromatic rings (Table S1), as these properties can influence their biodegradation by pure bacterial strains. Despite musks HHCB and AHTN sharing similar molecular weights and molecular properties, their degradation potentials differed between S-2, S-1, and S-3. The UV filters OC and OD PABA with different lengths and levels of chain branching exhibited various percentage removals by strains S-1, S-2, and S-3. There appears to be no clear trend in the degradation potential of strains based on the chemical composition of the pollutants. However, differences in degradation potential were observed between hydrophobic compounds (HHCB, AHTN, OC, OD PABA) and hydrophilic compounds (KTP, OXA, ATZ). It seems that hydrophilic compounds exhibit greater resistance to biodegradation than hydrophobic compounds. Specifically, among the hydrophilic compounds, only S-1 showed degradation capability for KTP, while *Rhodococcus* sp. was unable to degrade any hydrophilic substances. It is important to acknowledge that the concentrations utilized in toxicity tests far exceed the solubility threshold of the majority of hydrophobic compounds examined in this study. However, bacterial biomass can play a crucial role in enhancing the bioavailability of hydrophobic pollutants, even if these compounds are not soluble in the medium alone. There is evidence demonstrating a correlation between cell-surface hydrophobicity and the removal of hydrophobic organic compounds. Some bacteria with low cell surface hydrophobicity (CSH) develop resistance to solvents by changing the composition of their outer membrane and become efficient in the removal of pollutants [57]. In other studies, efficient degradation of pollutants was shown in bacteria with high level of CSH [58–60]. Although there is no evidence of the fate of CECs once degraded by the strains used in this study, some bacterial strains are known to render organic pollutants bioavailable, breaking them down and using them as a carbon source [26,27].

The results also suggest a positive correlation between marine bacterial resistance to CECs and their high biodegradation potential.

Strains S-1 and S-2 are resistant to and capable of efficiently degrading HHCb, AHTN and OC, supporting this correlation. The relationship becomes more apparent with the behavior of strain S-2 towards KTP. Indeed, Strain S-2 is not capable of removing KTP and is the most sensitive strain to this pollutant. However, this hypothesis may not apply to all pollutants, since strain S-2 was susceptible to and capable of degrading OD PABA, although slight inhibition of the strain by OD PABA was observed, but not complete even up to 500 mg L<sup>-1</sup>.

#### 4. Conclusion

The data presented in this work showed that bacterial strains of *Priestia* and *Rhodococcus* genera isolated from submarine sediments are resistant to and capable of removing several CEC compounds, especially those with a high hydrophobicity index. This finding may partially explain the higher resistance of some bacterial species to CECs when compared to marine eukaryotes, which could contribute to their survival and adaptation in CEC-contaminated environments. However, these observations may not be applicable to all marine bacteria tested thus far. While most bacterial strains demonstrated resistance to CECs, some, including those reported in the literature, exhibited sensitivity to these compounds at concentrations similar to those found in CEC-contaminated aquatic environments. Moreover, this study identified two bacterial strains, *Priestia* sp. 35 OD PABA G14 (strain S-1) and *Rhodococcus* sp. 23 AHTN G14 (strain S-2), with a high potential for the decomposition of multiple classes of CECs. These strains show promise for advancing bioremediation strategies against persistent CECs and other organic pollutants in the environment.

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#### CRediT authorship contribution statement

**Hatice Turan:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bahia Khalfaoui-Hassani:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **Alisson Godino-Sanchez:** Investigation, Formal analysis, Data curation. **Zulfatun Naimah:** Investigation, Formal analysis, Data curation. **Mathieu Sebilo:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Rémy Guyoneaud:** Resources, Data curation, Conceptualization. **Mathilde Monperrus:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

#### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve language and readability. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

#### Declaration of competing interest

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

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.emcon.2024.100332>.

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