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

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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⁻¹. Only Ketoprofen was toxic to bacterial cells, particularly to Rhodococcus sp. starting at concentration as low as 4 mg L⁻¹. 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]. Pharmaceutical 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⁻¹ to mg L⁻¹ [10]. The acute toxicity of musks such as galaxolide (HHCB) and tonalide (AHTN) to various aquatic organisms including Nitocra spinipes [11], Acartia tonsa [12], Lampsis cardium [13], Oryzias latipes [14], Scenedesmus quadricauda and Navicula sp. [15] was evaluated with their LC₅₀ ranging from 0.02 to 12 mg L⁻¹. Additionally, Danio rerio [16] and Pseudokirchneriella subcapitata [17] exhibited lowest observed effect...
rhodococci are resistant to CECs at concentrations greater than UV closely. Few studies have demonstrated that pharmaceuticals and communities, particularly marine bacteria, have not been studied of CECs on marine eukaryotes, their effects on marine microbial A.

varying degradation capacities for different CECs, with some being achieved by exploiting the catabolic activities of microbial consortia treatment plants (WWTPs), the biodegradation of CECs have been various CECs [30,31]. In engineered systems like wastewater communities in marine sediments have been reported to remove of organic pollutant biodegradation. For instance, natural microbial water treatments sludges, in order to understand the mechanisms 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, Nocardi, 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 IEMG 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 Cabreton canyon (Southwest of France) sediments ranging from 0.8 to 29.2 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 Cabreton 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 Cabreton 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 Cabreton 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.

Abbreviations

CEC(s) contaminant(s) of emerging concern
EHMC hexyl-4-methoxyccinnamate
4-MBC 4-Methylbenzylide-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 mg L⁻¹. Pharmaceuticals, such as ibuprofen, ketoprofen, novobiocin and carbamazepine, are also toxic and affect the behavioral and physiological parameters of the Mollusca Rudipatus philipinnum [18], the Annelida Hediste diversicolor [19], and the Arthropoda Daphnia magna [20], with concentrations as low as ng L⁻¹ to μg 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 mg L⁻¹, similar to the most resistant marine eukaryotes. However, other studies have shown that A. fischeri [23], Bacillus thuringiensis B1 [24] and Alkalotrophic rhodococci [25] are resistant to CECs at concentrations greater than 250 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,
2. Material and methods

2.1. CECs standard solutions

Different compounds were used for the experiment: HHCB from TCR (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, AXA, as classified by analytical grade (>98%), were also obtained from Sigma-Aldrich. The internal standards were Carbamazepine-d10, Nordiazepam-d5, Atarazine-d5 from Sigma-Aldrich and Musk Xylene-d15 from LG (MX-d15, 96.8% purity, 100 ng mL⁻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 °C.

Table S1 summarizes the main characteristics of the emerging contaminants used in this study namely hydrophobic (HHCB, AHTN, OC, OD PABA; log KOW between 5 and 7) and hydrophilic (KTP, OXA, ATZ; log KOW 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 (MM20) 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 contaminant 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 (T0) and final time (Tf) 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 acetonitrile overnight and subjected to pyrolysis.

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 L⁻¹. The CEC-containing solvents were evaporated in each well before bacterial inoculation. The freshly inoculated MM5 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 μL. The microplates were incubated at 30 °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 AOD, monitored at 600 nm, was calculated as ODₙₐₙₓ — ODₘᵢₘᵢₐₙ and the growth rate (μ) was determined using the formula: $\mu = \frac{\ln(N_t/N_0)}{t}$, where N₀ is the cell count at exponential phase, N₀ is the initial cell count, and t is the incubation time at N₀.

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 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 (T₀) and final time (Tₕ) 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 acetonitrile 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 μm polytetrafluoroethylene (PTFE) filter and transferred into vials. Finally, the samples were stored at -20 °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 ultrainert liner with glass wool and a HP-5MS UI capillary column (30 m length x 250 μm diameter x 0.25 μm 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 µg L⁻¹, as well as internal standard at 150 µg L⁻¹.

2.5.3. LC – MS/MS measurements

The concentration of KTP and OXA were determined by LC-MS/MS analysis. Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Bacterial strains</th>
<th>Compound used for isolation</th>
<th>Phylum</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>OD PABA G14</td>
<td>OD PABA</td>
<td>Bacillus (Firmicutes)</td>
<td>Priestia</td>
</tr>
<tr>
<td>S-2</td>
<td>23 AHTN G14</td>
<td>AHTN</td>
<td>Actinomycetota (Actinobacteria)</td>
<td>Rhodococcus</td>
</tr>
<tr>
<td>S-3</td>
<td>AHTN G14</td>
<td>AHTN</td>
<td>Bacillus (Firmicutes)</td>
<td>Bacillus</td>
</tr>
<tr>
<td>S-4</td>
<td>40 OC G14</td>
<td>OC</td>
<td>Bacillus (Firmicutes)</td>
<td>Bacillus</td>
</tr>
</tbody>
</table>

Table 1

Pure bacterial strains used in this study.

H. Turan, B. Khalfaoui-Hassani, A. Godino-Sanchez et al. Emerging Contaminants 10 (2024) 100332
The growth of strain S-1 (Fig. 1) of the same packing material. The 0.1% formic acid.

tracting the CECs concentration at the

musks (HHCB and AHTN), UV sending production as well as the calculated exponential growth

standard deviation.

The degradation percentages of CECs were calculated by subtracting the CECs concentration at the final time (CECTf) from the mean concentration at the initial time (CECT0, mean), dividing this by the mean concentration at the initial time (CECT0, mean) for each replicate using the following equation:

\[
\text{degradation of CEC} = \frac{\text{CECT}_0 \text{- mean} - \text{CECT}_f}{\text{CECT}_0 \text{- mean}} \times 100
\]

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

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 (CECTf) from the mean concentration at the initial time (CECT0, mean), dividing this by the mean concentration at the initial time (CECT0, mean) for each replicate using the following equation:

\[
\text{degradation of CEC} = \frac{\text{CECT}_0 \text{- mean} - \text{CECT}_f}{\text{CECT}_0 \text{- mean}} \times 100
\]

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 Priesta 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 mg L−1 (tested at concentrations from 2 to 60 mg L−1 and then from 100 to 500 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 ΔOD (ODmax - ODmin), representing production as well as the calculated exponential growth rate (μ expo).

3.1.1. S-1 and S-2 strains resist HHCB, AHTN, OC and OD PABA up to 500 mg L−1

In the absence of pollutants, the calculated growth rates were up to 0.45 and 0.09 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 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 mg L−1 (Fig. 1) (p-value <0.05). Although a decrease in the growth rate of S-2 was observed up to 60 mg L−1 for OD PABA, complete growth inhibition was not recorded, even up to 500 mg L−1.

Therefore, the S-1 (Priesta 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 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-methoxybenzincinnamate (EHMC) with EC50 values as low as 8.57 and 1.06 mg L−1, respectively [21]. Similarly, benzophenone (BP), 2-Hydroxybenzenophenone (2HB), and BP3, inhibit Aliivibrio fischeri growth with EC50 values ranging from 13 to 18 mg L−1 [23]. However, Aliivibrio fischeri shows resistance to 2-Hydroxy-4-methoxybenzophenone-5-sulfonicacid (BP4) with an EC50 as high as 858.95 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 (Priesta sp.), this UV filter partially inhibits the growth of S-2 (Rhodococcus sp.) up to 60 mg L−1, with no complete inhibition seen even up to 500 mg L−1. Currently, there are no studies on the effects of OD BAPA 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 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 mg L−1, while the growth of strain S-2 was inhibited starting from 2 mg L−1, reaching complete inhibition at 30 mg L−1. Significant inhibition values were observed starting at 30 mg L−1 for strain S-1 and 4 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 EC50 ranging from 1 to 5 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 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 mg ml−1 to 500 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 Priesta and Rhodococcus genera isolated from the Cabreton canyon sediments. Notably, this resistance is particularly evident for CECs with a
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 > 0.05). Notably, S-1 (p-value < 0.001), S-2 (p-value 0.018 < 0.05), and S-3 (p-value 0.060 > 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 ± 4% to 97 ± 1% for HHCB, AHTN, and OC, and 52 ± 11% for OD PABA. In comparison, S-2 demonstrated degradation percentages of 14 ± 11%

![Fig. 1. The growth rate (µ expo) and cell production (ODmax – ODmin) 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.](image-url)
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\(^{-1}\) [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 desulfurization, dehalogenation, and hydroxylation of aromatic and aliphatic compounds. The monoxygenase 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 hydrophobic 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.

Fig. 2. Degradation percentage of hydrophobic compounds (musks; HHCB and AHTN, UV filters; OC and OD PABA) using selected isolated strains (Table 1). Abiotic control (control) was performed without inoculum in medium MM20. Initial exposure concentration was 1 mg L\(^{-1}\). Data are mean ±SD of three replicates.

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 MM20. Initial exposure concentration was 1 mg L\(^{-1}\). Data are mean ±SD of three replicates.
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⁻¹.

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

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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

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References
