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RESEARCH ARTICLE

Identification and expression of MarCE, a marine carboxylesterase with synthetic ester-degrading activity

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Abstract

Carboxylic ester hydrolases with the capacity to degrade polyesters are currently highly sought after for their potential use in the biological degradation of PET and other chemically synthesized polymers. Here, we describe MarCE, a carboxylesterase family protein identified via genome mining of a *Maribacter* sp. isolate from the marine sponge *Stelligera stuposa*. Based on phylogenetic analysis, MarCE and its closest relatives belong to marine-associated genera from the Cytophaga–Flavobacterium–Bacteroides taxonomic group and appear evolutionarily distinct to any homologous carboxylesterases that have been studied to date in terms of structure or function. Molecular docking revealed putative binding of BHET, a short-chain PET derivative, onto the predicted MarCE three-dimensional structure. The synthetic ester-degrading activity of MarCE was subsequently confirmed by MarCE-mediated hydrolysis of 2 mM BHET substrate, indicated by the release of its breakdown products MHET and TPA, which were measured, respectively, as 1.28 and 0.12 mM following 2-h incubation at 30°C. The findings of this study provide further insight into marine carboxylic ester hydrolases, which have the potential to display unique functional plasticity resulting from their adaptation to complex and fluctuating marine environmentsw.

INTRODUCTION

The use of enzymes to facilitate 'greener' chemical manufacturing processes together with their use in the biodegradation of synthetic waste has progressed significantly in recent decades, with a particular recent focus on obtaining highly efficient biocatalysts via sequence database search algorithms and computer-guided protein design (Mican et al., 2024; Tournier et al., 2023). Even so, it remains important to sustainably 'mine' natural ecosystems for new biocatalysts and to consider the environmental or microbial

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origins of a given enzyme, which can often provide clues regarding its biochemical properties and native ecological function (Ogawa & Shimizu, 1999; Reen et al., 2015). Although marine ecosystems have become increasingly valued as a source of microbial biocatalysts, marine microorganisms and their enzymes are still relatively underutilized from a biotechnological standpoint, particularly when compared to their terrestrial counterparts (Rotter et al., 2021). Nonetheless, efforts to characterize marine microbial activities have to date proven worthwhile, leading to an increased diversity in the pool of available biocatalysts and the identification of enzymes with novel properties including high salt tolerance, temperature adaptivity, barophilicity and unique substrate specificities (Gavin et al., 2019; Rodrigues et al., 2017; Trincone, 2017). In particular, microbial enzymes produced by microorganisms isolated from marine sponges are rarely reported on, but represent a potentially promising biodiscovery resource, especially considering the important role they play in the degradation of organic matter that is extracted by the sponge from the large quantities of seawater being pumped through its filter-feeding structures for energy and nutrient gain (de Oliveira et al., 2020; Wang, 2006).

The class of enzymes termed carboxylic ester hydrolases (CEHs, EC 3.1.1.x) represents a wide range of proteins that are ubiquitously distributed across living systems and that are known to act on ester and ester-linked substrates (Bornscheuer, 2002). Microbial CEHs tend to be highly diverse in terms of their biological roles, with potential involvement in various cellular interactions, metabolic processes and signalling pathways (Oh et al., 2019). This multifunctionality can be leveraged for biocatalysis, biodegradation and biotransformation purposes, where alternative or non-native substrates can be processed by these enzymes. In particular, the degradation of synthetic polyesters by microbial CEHs has been explored with the aim of developing systems for the biorecycling or bioremediation of polyester-based plastic materials (Urbanek et al., 2020). Polyester-degrading enzymes, collectively referred to as 'polyesterases', include CEHs homologous to carboxylesterases (CEs), lipases and cutinases. Cutinase-like polyesterases have been noted as the most efficient degraders of recalcitrant polyesters like polyethylene terephthalate (PET), but dual enzyme-, synergistic cocktail- or multienzyme cascade-type strategies have been proposed to further promote the hydrolysis of waste plastic (Gricajeva et al., 2022).

Marine microbial CEHs have been identified and characterized, including examples from *Pseudoalteromonas*, *Vibrio*, *Bacillus*, *Erythrobacter*, *Pseudomonas* and *Oleispira* species together with those from metagenomic sources (Lee et al., 2010; Rodrigues et al., 2017). In a previous study of marine metagenomic libraries prepared from crude oil-contaminated seawater enrichment cultures, five cold-active, salt-tolerant carboxylesterases exhibited broad substrate profiles, efficiently hydrolysing a range of mono- and polymeric esters (Tchigvintsev et al., 2015). More recently, a carboxylesterase named E93 was identified from a marine strain of the bacterium *Altererythrobacter indicus* and investigated in terms of specificity towards CPT11 and NPC ester prodrug substrates, together with *p*-nitrophenyl esters; expanding the knowledge of hydrolysis of such compounds by microbial enzymes (Li, Rong, et al., 2023). In addition, marine-derived bacterial CEHs such as Ple628 and Ple629 (Meyer Cifuentes et al., 2022), PE-H (Bollinger et al., 2020) and SM14est (Carr et al., 2023) have been shown to hydrolyse PET among other polyesters, as reported in the plastics-active enzyme database (PAZy) (Buchholz et al., 2022). Based on a metagenomic study, genes encoding PET hydrolase homologues in marine-associated ecosystems are most commonly detected in the phylum *Bacteroidota* (Danso et al., 2018) and two bacteroidetal PET-active enzymes, PET27 and PET30 were recently characterized in detail (Zhang et al., 2022).

Here, we report on a carboxylesterase enzyme named MarCE, which was found encoded in the genome of a marine sponge-derived *Maribacter* sp. isolate. Comparative analyses of MarCE with homologous carboxylesterases that had previously been shown to degrade short-chain oligomers of polyethylene terephthalate (PET) and putative docking of the PET derivative bis(2-hydroxyethyl) terephthalate (BHET) revealed key structural and catalytic components. The MarCE protein features a hydrolase domain carrying Ser-His-Glu triad residues and a Gly-Gly oxyanion motif, together with two lid domains that are thought to mediate substrate specificity. MarCE was heterologously expressed in *Escherichia coli* and confirmed to possess BHET-hydrolysing activity. A general overview of the work carried out with respect to MarCE is presented in Figure 1.

EXPERIMENTAL PROCEDURES

Identification and classification of MarCE

The *Maribacter* sp. strain (sample ID: J2146c) used in this study was previously isolated from the sponge *Stelligera stuposa* sampled at Lough Hyne, Cork, Ireland (Jackson et al., 2012) and later selected for whole-genome sequencing based on its hydrolytic activities observed towards tributyrin, polycaprolactone diol (PCD) and polycaprolactone (PCL) substrates, similar to the approach we recently employed to identify

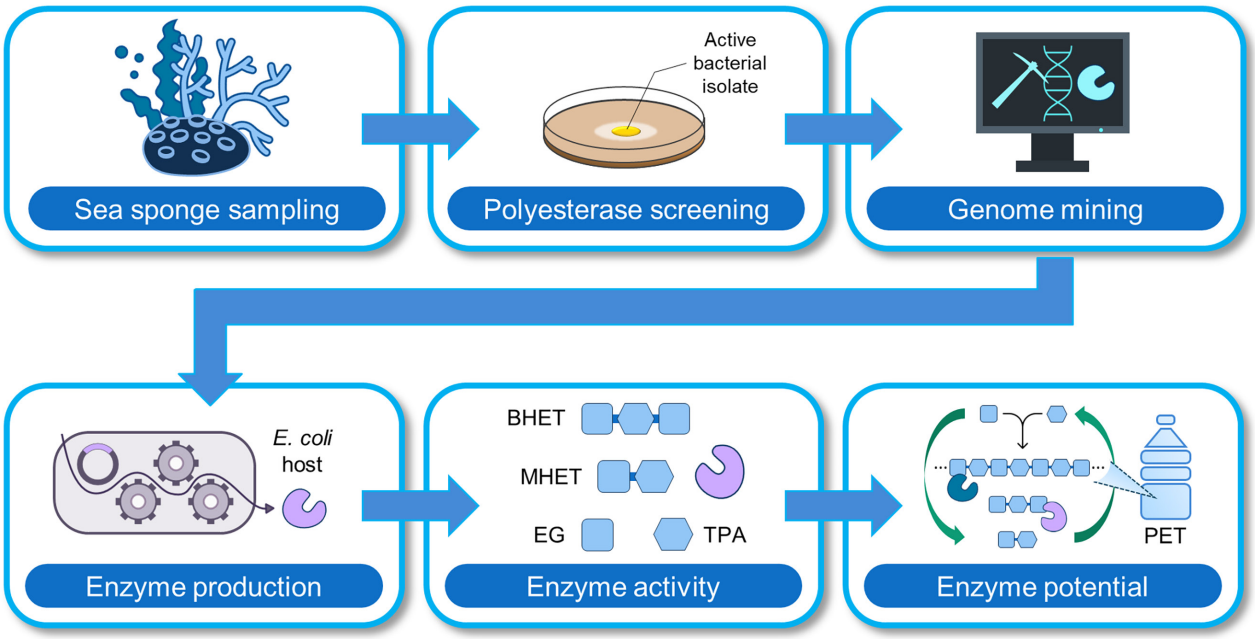

FIGURE 1 Visual representation of the workflow employed to identify MarCE and verify its BHET-hydrolysing activity. The marine sponge-derived *Maribacter* sp. isolate was detected as a potential polyesterase producer during screening. Its' genome was mined for polyesterase-encoding genes and an enzyme designated MarCE was predicted to bind BHET prior to its expression in *Escherichia coli* and subsequent activity confirmation towards BHET, a short-chain derivative of the synthetic polymer PET.

the polyesterase BgP (Carr et al., 2022) (Procedure 2.1 in Data S1).

Genomic DNA (gDNA) was extracted with the QIAamp® DNA Mini Kit (Qiagen) from 5-mL cultures grown by shaking for 24 h in Marine Broth 2216 at 28°C. Illumina HiSeq sequencing (NovaSeq 6000 system), library preparation and initial quality control were performed by Eurofins Genomics (Konstanz, Germany). The Genome Taxonomy Database Toolkit (GTDB-Tk, v 1.5.0) was used to classify the isolate J2146c (Chaumeil et al., 2020). The sequence reads were assembled by SPAdes (v 3.15.0) (Bankevich et al., 2012) and initial annotation was performed using Prokka (v 1.14.6) (Seemann, 2014), while eggNOG mapper (v 2.0) facilitated functional annotation (Huerta-Cepas et al., 2019) (Table S1).

The *Maribacter* sp. genome was mined for homologues of known polyester-degrading enzymes using a custom BLASTP database constructed from reference sequences using the makeblastdb command-line tool (Table S2). MarCE was identified as a homologue of the carboxylesterase BsEstB. It should be noted that while BsEstB was originally demonstrated to hydrolyse PET films (120-h reaction) (Ribitsch et al., 2011), a more recent study concluded that the *Bacillus subtilis* carboxylesterase performs poorly on polymeric PET following comparative kinetic analysis with three cutinase-like enzymes from *I. sakaiensis*, *H. insolens* and *T. fusca* (24-h reaction) (Bååth et al., 2021). In the same study, the *B. subtilis* carboxylesterase was, however, shown to outperform these three PET-degrading enzymes in terms of activity towards BHET as a substrate. Pfam, InterProScan and SUPERFAMILY web tools were used for protein classification of MarCE, while the SignalP 5.0 server was used to predict the native MarCE signal peptide sequence, which was subsequently removed (Almagro Armenteros et al., 2019).

Phylogenetic and structural protein analyses

MEGA-X and MUSCLE software were employed for phylogenetic analysis of MarCE and 46 carboxylesterase family homologues (Kumar et al., 2018) (Table S3). Alpha-Fold2 (MMseqs2 mode, v1.3.0) was run via ColabFold to predict the structure of MarCE (Mirdita et al., 2022) (Figure S1). The model was visualized in UCSF Chimera and the Chimera MatchMaker tool was used to facilitate structural comparison with homologues of interest (Pettersen et al., 2004). Protein structural alignments were generated by T-COFFEE Expresso (Di Tommaso et al., 2011) and graphically rendered using ESPript 3.0 (Gouet et al., 1999) (Figure S2). AutoDock Vina (v 1.1.2) was employed to dock BHET onto MarCE, with the search box set around the centre of the catalytic triad (edge length = 15 Å, exhaustiveness = 8). The binding modes were visualized using ChimeraX and BIOVIA Discovery Studio. The best-scored pose of BHET (Figure S3) was used as initial geometry for molecular mechanics optimization of the

MarCE:BHET complex in explicit solvent (Procedure 2.2 in Data S1).

Heterologous expression and activity confirmation

The *marCE* gene was amplified from gDNA and cloned into a pET20b(+) plasmid (Novagen®) and the resulting construct was conjugated into *E. coli* BL21-Codon Plus (DE3)-RIPL (Agilent Technologies) (Figure S4). Fresh cultures of the pET20b:MarCE clone and an empty vector negative control were induced with 0.5 mM IPTG (Melford) for 20 h at 20°C. The cultures were pelleted by centrifugation (17,700 × *g*) for 10 min at 4°C (Beckman Coulter, Avanti JXN-26, JA-10 rotor). The resulting supernatants were analysed by SDS-PAGE (10% resolving gel, 5% stacking gel, tris-glycine-SDS running buffer and 2X β-mercaptoethanol sample buffer) and polyesterase activity was tested on PCD (1%) agar (Figure S5). MarCE-containing supernatant and the negative control supernatant were stored at −70°C prior to further activity analysis.

To assess BHET hydrolysis, supernatants (75 μL) were incubated with a 2 mM BHET (Sigma Aldrich) suspension and 25 mM Tris–HCl (pH 7.5) in a final volume of 1 mL at 30°C and 1000 rpm. Reactions were performed in triplicate, with samples taken at 30 min, 1 h and 2 h, then quenched by centrifugation (17,000 × *g*) and the addition of an equal volume of 200-mM sodium phosphate buffer (pH 2.5, 20% DMSO) to the supernatant, with samples heat treated at 95°C prior to storage at −20°C. The resulting samples were analysed by UHPLC using an Agilent 1260 Infinity II LC System and a Kinetex® 5 μm EVO C18 100 Å column, with formic acid and acetonitrile employed during the injection of 3-μL samples. The analytes were separated using a flowrate of 1 mL/min for 5 min, where the retention times of terephthalic acid (TPA), mono(2-hydroxyethyl) terephthalate (MHET) and bis(2-hydroxyethyl) terephthalate (BHET) were 2.4 min, 2.6 and 3.1 min respectively. The concentrations of TPA, MHET and BHET were determined against a standard curve of TPA (0–1 mM).

RESULTS AND DISCUSSION

Petroleum-based, non-biodegradable synthetic plastics have an environmental half-life that ranges from several to a few thousand years. Since PET plastic is employed in packaging and textiles due to its strength and physicochemical resistances, PET-based materials are slow to degrade in nature, whether by microbial-based or abiotic processes (Li, Menegatti, & Crook, 2023). The conversion of waste plastics into high-purity monomers or value-added products has gained much traction as a way to sustainably manage and circularize the life cycle of synthetic materials, with biocatalytic approaches of particular interest to enable depolymerization under mild reaction conditions (Lee et al., 2023). The biological recycling and upcycling of PET is viable due to the capacity of polyesterases to break the ester bonds that link each polymer subunit (Wei et al., 2022). Polyester-hydrolysing biocatalysts are innately promiscuous and are also potentially useful for the bioconversion of organic compounds to yield valuable pharmaceutical intermediates (Biundo et al., 2019; Sanchez & Demain, 2011).

Following enzymatic activity screening, a *Maribacter* sp. strain isolated from a *Stelligera stuposa* sponge sampled from the marine waters of Lough Hyne was noted as a promising polyesterase producer. Its closest relative was determined to be *Maribacter litoralis* using the GTDB-Toolkit and a high-quality draft genome was assembled, which displayed similar properties to previously deposited *Maribacter* spp. genomes. Using an E-value cut-off of 1e-30, the *Maribacter* sp. protein-coding sequences were mined for polyesterases against a custom database composed of functionally verified examples, with the top hit producing a significant alignment with the reference sequence BsEstB. T-Coffee Expresso alignment revealed a mean amino acid identity of 30.5% between their sequences and a mean similarity of 72.9%.

The predicted *Maribacter* sp. protein was initially annotated by Prokka as a 'fumonisin B1 esterase', but a general NCBI BLASTP search against bacterial entries in the Swiss-Prot database revealed similar sequence identities to other members of the carboxylic ester hydrolase (CEH) class of enzymes (Table S4). Based on eggNOG mapper, InterProScan, Pfam and SUPERFAMILY outputs, the enzyme was broadly classified as an α/β hydrolase belonging to the carboxylesterase (CE, EC 3.1.1.1) family, and was designated as MarCE. According to the Arpigny and Jaegar classification scheme for microbial carboxylesterases, MarCE belongs to the carboxylesterase family VII (Sood et al., 2016). A wider search against the NCBI's non-redundant database showed that the closest homologues of MarCE are other *Maribacter* spp. carboxylesterase family proteins, which share sequence identities of up to 97.7% but have not, as of yet, been further studied or biochemically characterized in terms of their activity or substrate range (Table S4).

The MarCE-producing *Maribacter* sp. strain was isolated from a marine sponge sampled from Lough Hyne, which is a salt-water lake connected to the Celtic Sea of the Atlantic Ocean by a narrow tidal channel (Plowman et al., 2020). As Europe's first Marine Nature Reserve, Lough Hyne is regarded as a biodiversity hotspot, with a notable abundance of sponge assemblages across its individual habitats that display comparable species richness to tropical, polar and temperate sites for which similar data are available. This fully marine lake

is subjected to an unusual, but predictable tidally generated flow regime that is restricted compared to that of the open Atlantic coast, which is thought to contribute to the observed sponge diversity and abundance patterns (Bell, 2007). *Stelligera stuposa* is a species of demosponge with robust branching morphologies that facilitate enhanced feeding and adaptation, for example, to current flow (Bell et al., 2002).

The Genus *Maribacter*, which belongs to the family Flavobacteriaceae of the Phylum Bacteroidota, was first described in 2004 and currently consists of 30 validated species (according to the LPSN collection, accessed December 2023) (Nedashkovskaya et al., 2004). *Maribacter* species appear to be exclusively associated with marine environments, including a diverse range of isolates having been identified from seawater (Yoon et al., 2005), algae (Zhang et al., 2020), sponge (Jackson et al., 2015), deep-sea sediment (Fang et al., 2017), seaweed (Weerawongwiwat et al., 2013), Arctic (Cho et al., 2008) and Antarctic (Zhang et al., 2009) sources. To date, the majority of published work on the genus *Maribacter* has been related to the description of new species, the deposition of genomic sequences or their interactions with macroalgae, but with only a small number of *Maribacter* spp. enzymes being specifically characterized, primarily for their ability to degrade polysaccharides (Lee et al., 2016; Lu et al., 2022). To our knowledge, there are no reports describing other types of enzymes from *Maribacter* species, for example, those with activity towards ester and polyester substrates.

Phylogenetic comparison of MarCE with a selection of protein homologues suggests some evolutionary divergence between *Maribacter* spp. carboxylesterase-type enzymes and their closest functionally characterized relatives, as indicated by the separation of the resulting consensus tree into two distinct clades (labelled 1 and 2, Figure 2). The first clade features carboxylesterases from CFB (Cytophaga, Flavobacterium, Bacteroides) group bacteria, which are associated with the phylum Bacteroidota and that are considered to be important contributors to the degradation of marine organic matter (Brettar et al., 2004). The second clade features reference carboxylesterases (i.e. those sourced from Swissprot or PDB databases for which experimental or structural data is available, or that have been previously studied in the literature).

MarCE is found in clade 1a with proteins from *Maribacter litoralis* (marine sediment-derived strain) and *Maribacter caenipelagi* as its closest relatives, while sister groups are formed primarily by entries from other members of the *Maribacter* genus but also featuring sequences from *Saonia flava* of the Flavobacteriaceae Family and *Fulvivirga sedimenti* of the class Cytophagia (Figure 2). The clade 1b consists of Flavobacteriaceae sequences exclusively belonging to marine-associated Genera (*Pricia*, *Eudoraea*, *Euzebyella* and *Maribacter* spp.) (Siamphan et al., 2015; Yu et al., 2012; Zhang et al., 2017) or in one case, coming from a marine-derived metagenomic sample. The proteins in clade 1c each belong to the Cytophagia class of bacteria, featuring genera that are not specific to the marine environment (*Arcicella*, *Emticicia* and *Runella* spp.), which are instead associated with terrestrial, freshwater or wastewater samples (Nikitin et al., 2004; Ten et al., 2019; Yang et al., 2020). The carboxylesterases clustered in clade 2 of the phylogenetic tree represent publicly available reference sequences, which share relatively low levels of homology (<35% amino acid identity) with MarCE, but which have been previously studied in the context of their function and/or structure. Protein sequences from the species *Thermobifida fusca* and the family *Bacillaceae* form 2a and 2b, respectively, while the remainder are assigned to the Phyla Actinomycetota (*R. erythropolis* and *P. oxydans*), Alphaproteobacteria (*S. macrogoltabida* and *A. indicum*) or Bacillota (*H. hathewayi*). TfCa from *Thermobifida fusca* KW3 (clade 2a) has proven useful as a secondary biocatalyst for polyester degradation in combination with PET hydrolases such as TfCut2 and LC-cutinase (Barth et al., 2016).

Microbial enzymes which have been shown to depolymerize PET and other polyesters, such as polycaprolactone, PBSA (poly(butylene succinate-co-adipate)), PBAT (polybutylene adipate terephthalate) and polylactic acid (Hajighasemi et al., 2016; Li, Rong, et al., 2023; Wu et al., 2023), may be broadly classified as carboxylic ester hydrolases (CEHs, EC 3.1.1.x). Of these, cutinase family members (EC 3.1.1.74) are currently considered the most efficient degraders of PET; however, the associated reaction does not typically result in the full hydrolysis of the polyester to its constituent monomers (i.e. terephthalic acid (TPA) and ethylene glycol (EG)) and instead, there tends to be an accumulation of MHET and BHET intermediates which are not as easily depolymerized by cutinases (Schubert et al., 2023). By comparison, members of the carboxylesterase family (CEs, EC 3.1.1.1) exhibit poor activity towards polymeric PET but seem to be more accommodating of shorter PET fragments (Bååth et al., 2021).

BsEstB and TfCa are two carboxylesterases known to act on PET oligomers. While MarCE shares slightly higher amino acid similarity values to BsEstB than it does with TfCa (72.90% vs. 70.76%), the latter has been studied in greater detail as a (tere)phthalate-ester hydrolase that acts on oligo-ester substrates (von Haugwitz et al., 2022). Thus, TfCa from *Thermobifida fusca* was selected for comparison with MarCE. Following structure-based amino acid sequence alignment, MarCE was found to share a mean similarity of 70.76% with TfCa and a mean identity of 27.08% (Figure S2). Based on its characterization, TfCa is not considered to be active on PET alone, but this carboxylesterase can promote PET hydrolysis in combination

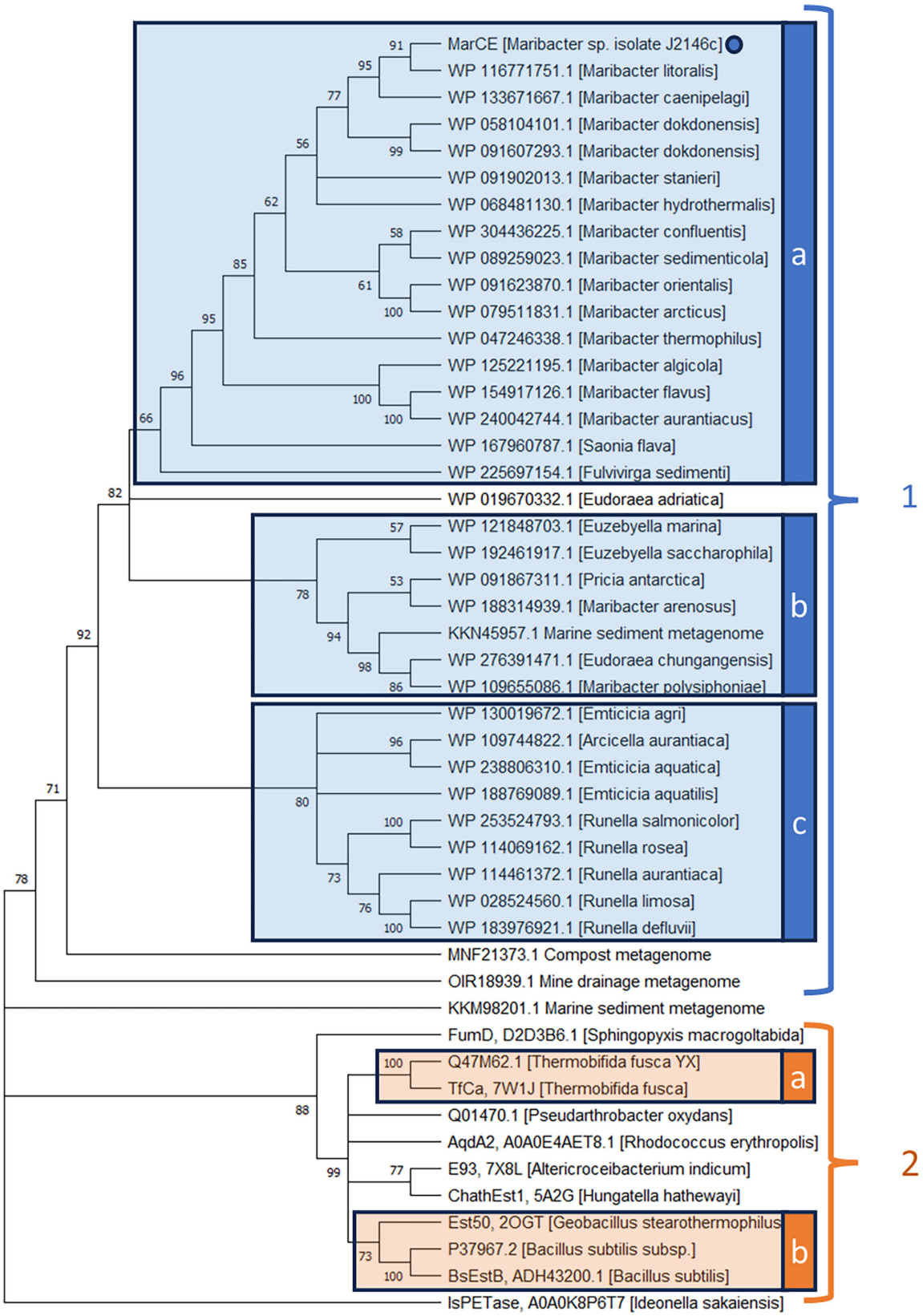

FIGURE 2 Phylogenetic consensus tree generated from the protein sequences of MarCE and 46 homologous carboxylesterase-like enzymes, rooted with IsPETase (NCBI accession: A0A0K8P6T7) as an outgroup. The maximum-likelihood method was employed to infer probabilities, with bootstrap values above 50% displayed on the tree for 100 replicates. The two main clades are numbered 1 and 2, within which smaller clades are highlighted in blue (1a, 1b and 1c) and orange (2a, 2b) respectively. The MarCE sequence is marked by a blue circle.

with PET-degrading enzymes and substrate profiling has revealed that TfCa is active on MHET and BHET (i.e. the breakdown products of PET), as well as 3PET (bis[2-(benzoyloxy) ethyl]) terephthalate and DEP (diethyl phthalate) oligomers (von Haugwitz et al., 2022). Therefore, we do not expect MarCE to display any significant activity towards PET, and we have instead investigated MarCE as a potential terephthalate-ester hydrolase.

During its characterization, TfCa was demonstrated to degrade BHET, among other short-chain synthetic ester substrates (von Haugwitz et al., 2022). Molecular docking of BHET as a ligand onto MarCE demonstrated putative binding (−5.8 kcal/mol), whereby the substrate fits comfortably into a pocket region on the enzyme's surface (Figure S3). The molecular mechanics of the MarCE:BHET complex were optimized and the residues of the main substrate binding pocket of MarCE were deduced, with the nucleophilic Ser198, the oxyanion hole-forming Gly113 and the amphoteric His432 present for ester bond hydrolysis (Figure 3A). Based on comparison with TfCa, there are differences in some key residues at the same position, for example, Phe73, Gly113, Ile202 and Phe277 in MarCE equates to Ile69, Ala108, Met189 and Leu282 in TfCa respectively (Table S5).

The superimposition of the MarCE predicted structure onto the elucidated crystal structure for TfCa revealed a similar overall structure for these two enzymes consisting of a core α/β hydrolase domain and two lid domains (Figure 3B). The hydrolase portion of MarCE features a mixed β-sheet composed of 12 full strands, seven of which run parallel in the centre with inversion at either end where the structure runs in anti-parallel. In general, anti-parallel β-sheets are thought to contribute increased flexibility over the parallel conformation (Emberly et al., 2004). Structural and spatial differences were observed between the two enzymes, for example, in the helical lid domains and in variable loop regions. The loops carrying the catalytic triad and oxyanion residues are more highly conserved, as is generally the case for α/β hydrolase superfamily members (Mindrebo et al., 2016). In general, the functionality and substrate range of each CEH depends on the structural configuration of their catalytic centres and the individual amino acids contributing to substrate binding and catalysis, with minor differences in sequence often having a major effect on activity for highly similar enzymes and variants (Leitão & Enguita, 2021; Ribitsch et al., 2017). Given that MarCE is derived from a marine mesophilic organism and TfCa is a thermophilic enzyme of terrestrial origin, differences in activity and substrate scope are likely to be observed upon functional comparison of the two enzymes (Crnjar et al., 2023).

Following heterologous expression by *E. coli*, MarCE (58 kDa) was detected from culture supernatant by

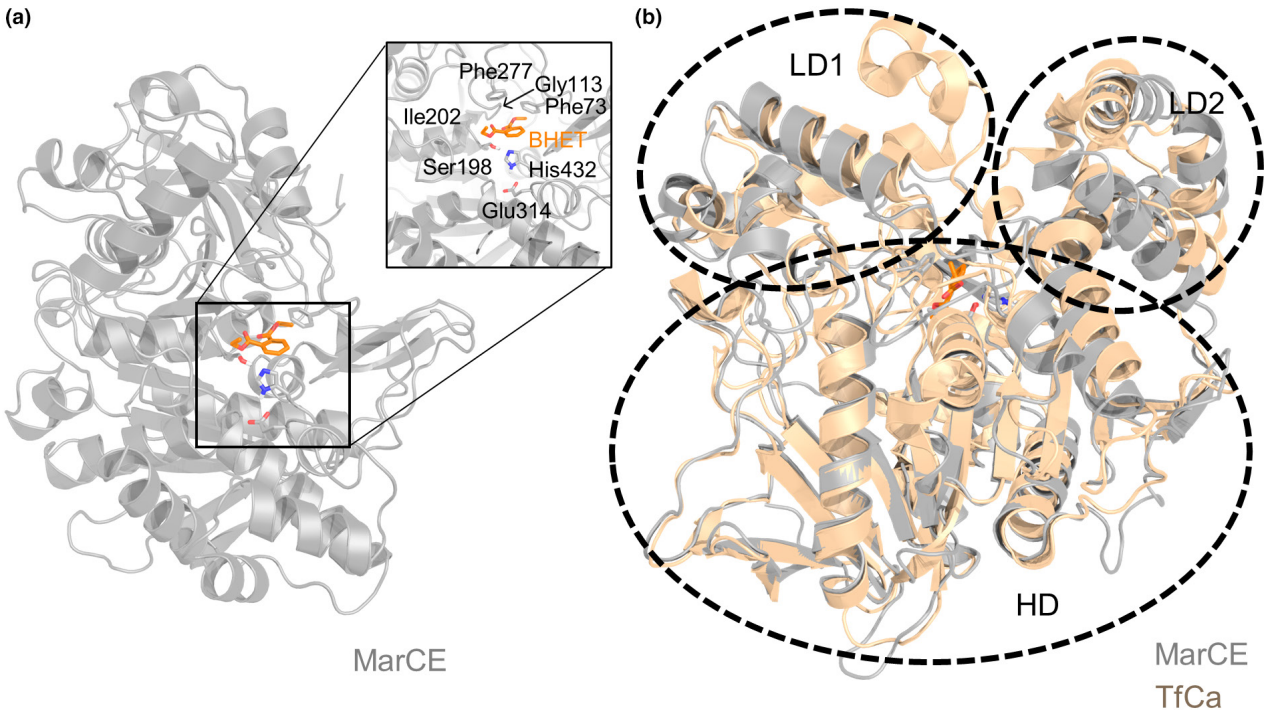

FIGURE 3 Structural model of MarCE bound to BHET. (A) Optimized 3D model for MarCE (C-atoms coloured in grey, shown as cartoon) in complex with BHET (C-atoms coloured in orange, shown as sticks). The active site is enlarged to allow better visualization of the residues of the catalytic triad (Ser198, His432 and Glu314). (B) Structural superimposition of the novel MarCE (in grey) onto the homologous carboxylesterase TfCa (PDB code: 7W1J, in beige). The lid domains (LD1 and LD2) and the hydrolase domain (HD) are circled to indicate their general position within each enzyme structure.

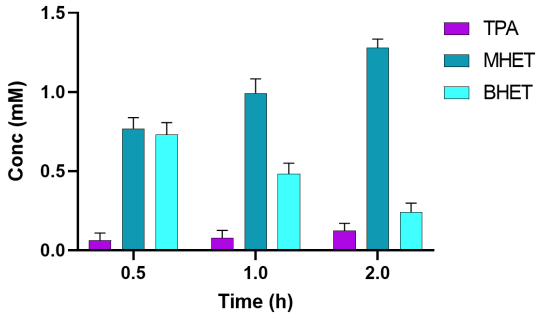

FIGURE 4 MarCE-mediated hydrolysis of BHET (2 mM) substrate, as indicated by the detection of BHET (cyan), MHET (teal) and TPA (magenta) following 30-min, 1-h and 2-h incubation at 30°C and subsequent UHPLC analysis. The 2-h reaction yielded 1.28 mM MHET and 0.12 mM TPA as the products of BHET degradation.

SDS-PAGE and the enzyme preparation, which was found to be relatively pure, was confirmed as active on PCD substrate plates (Figure S5). The MarCE-containing supernatant displayed BHET-hydrolysing activity at 30°C, with levels of available BHET decreasing over time (Figure 4). MHET was detected as the primary product of BHET hydrolysis by MarCE, where MHET levels were shown to increase with time. In addition, further degradation of MHET into monomers was observed, as indicated by the detection of TPA, which again increased over time. For the empty vector negative control sample, no conversion of BHET to TPA was observed (data not presented). The study of enzymatic PET degradation has been largely focused on cutinase homologues, which can accommodate and process polymeric PET, while the complementary role of carboxylesterases and their diversity have been explored to a lesser extent (Gricajeva et al., 2022; von Haugwitz et al., 2022; Wu et al., 2023). Thus, the degradation of PET derivatives by the marine carboxylesterase MarCE offers new insight into synthetic ester hydrolysis in general, and particularly within the unique context of the marine environment. Given that MarCE was shown to liberate TPA monomers from BHET, this enzyme could potentially be used in combination with PET-active polyesterases to facilitate the complete degradation of PET into its simplest constituent units.

CONCLUSION

In this work, we present the marine-derived carboxylesterase MarCE and its terephthalate ester-degrading activity, as demonstrated by the hydrolysis of BHET, an intermediate breakdown product of the polyester PET. Taking into consideration the true marine origin of this *Maribacter* sp. enzyme, combined with its overall homology to functionally characterized carboxylesterases, we believe that MarCE represents a novel addition to the current selection of microbial enzymes of interest for synthetic polyester degradation and the sustainable catalysis of organic synthesis reactions. Furthermore, the identification of MarCE contributes to our overall knowledge of marine carboxylic ester hydrolases and this enzyme may find use as a versatile biocatalyst for use in green chemistry applications. Finally, *Maribacter* species may represent a promising source of novel CEHs that are distinctly adapted to the marine environment, whose discovery could help to expand and diversify the selection of enzymes available for biological recycling among other biocatalytic applications.

AUTHOR CONTRIBUTIONS

Clodagh M. Carr: Conceptualization; formal analysis; investigation; project administration; writing – original draft. **Frederike Göttsch:** Investigation; writing – review and editing. **Bruno Francesco Rodrigues de Oliveira:** Conceptualization; writing – review and editing. **Pedro A. Sánchez Murcia:** Conceptualization; writing – review and editing. **Stephen A. Jackson:** Conceptualization; writing – review and editing. **Ren Wei:** Conceptualization; writing – review and editing. **David J. Clarke:** Conceptualization; writing – review and editing. **Uwe T. Bornscheuer:** Conceptualization; writing – review and editing. **Alan D. W. Dobson:** Conceptualization; funding acquisition; project administration; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available upon reasonable request to the corresponding author. The raw genomic sequence data has been deposited in the NCBI SRA database under the project code PRJNA1068038 and the gene sequence encoding MarCE has been deposited in Genbank under the accession code PP196553.

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SUPPORTING INFORMATION

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