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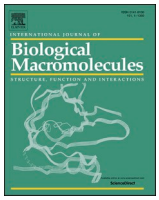

Identification of a PET hydrolytic enzyme from the human gut microbiome unveils potential plastic biodegradation in human digestive tract

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ABSTRACT

Widespread use of polyethylene terephthalate (PET) plastics and their recycling challenges have led to substantial accumulation of PET wastes in global environments, with inevitable consequences for their entry into the food chains. Recent studies have increasingly documented the ingestion of microplastics by humans through food and beverages. However, the fate of these microplastics within the gastrointestinal tract, particularly the role of the human gut microbiota, remains inadequately understood. To address this knowledge gap, we employed a bioinformatics workflow integrated with functional verification to investigate the PET digestion/degradation capabilities of intestinal microorganisms. This approach identified a novel PET hydrolase—HGMP01 from the human gut metagenome, which exhibits the capacity to hydrolyze PET nanoparticles. Moreover, comprehensive exploration for HGMP01 homologues in the human gut metagenome and metatranscriptome unveil their distribution in diverse intestinal microorganisms. This study provides biochemical evidence for an unforeseen role of human gut microbiome in plastic digestion, thus holding substantial implications for human health.

1. Introduction

Plastics are extensively used in industrial, medical, and agricultural sectors, and daily life owing to their extraordinary material properties and advantageous characteristics such as lightweight, corrosion resistance, and cost-effectiveness [1]. Global plastic production has increased continuously and approached 400 million tons per year, which is projected to double by 2040 [2,3]. However, the inherent durability of plastics leads to ubiquitous accumulation of plastic wastes in soils, freshwater, and oceans, which is considered as one of the most serious environmental and public health issues [4,5]. Plastic wastes in natural environments will experience a degradation process caused by the combination of atmospheric factors, such as waves, abrasion, ultraviolet radiation and photooxidation, as well as biological processes, which lead to the formation of microplastics (MPs) (1 μm – 5 mm) and nanoplastics (NPs) (<1 μm) [6,7]. Recent studies have indicated that organisms such as Antarctic krill [8] and rotifers [9] can significantly accelerate the fragmentation of MPs into NPs.

The ubiquitously distributed MPs in the environment eventually enter the global food chains and cause the inevitable exposure of animals and humans [10]. Recent studies have revealed the presence of MPs in the intestines and stomachs of marine and terrestrial animals [11–13]. In particular, MPs were detected in human feces for the first time in 2019 [14]. Human exposure to MPs occurs through food ingestion, air inhalation, and dermal contact, with ingestion as the primary route [15–17]. Once entering the human gastrointestinal tract, these plastic particles may enter the internal circulation system through the digestive system, leading to the appalling occurrence of MPs in human blood [6], thrombus [18] and breast milk [19]. The potential health damage caused by the internalization and accumulation of MPs in the human body is a problem that needs more attention. Despite limited knowledge of MPs impact on human health, several studies have reported significant toxic effects in various animal models and human cell lines [20–22]. By analyzing the characteristics of MPs in the feces of patients with inflammatory bowel disease (IBD) and healthy people, a significantly higher concentration of MPs was observed in the feces of

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IBD patients comparing to healthy individuals [10]. Moreover, NPs exhibit the capacity to traverse the blood-brain barrier, interacting with protein fibers within neurons and consequently exacerbating the risk of Parkinson's disease [23].

Polyethylene terephthalate (PET) is one of the most widely used packaging materials for beverages and caused serious pollution to the global environment. It is also one of the most prevalent types of plastic found in the human body [6,18,19]. Moreover, PET has garnered increasing attention in plastic degradation research due to its role as a substrate directly hydrolyzed by microbial enzymes, particularly since the discovery of *Is*PETase from *Ideonella sakaiensis* [24,25]. Given the abundance of microorganisms inhabiting the human digestive system, particularly within the intestinal tract, it is reasonable to hypothesize that this system may play a critical role in PET hydrolysis. Simulated gastrointestinal digestion demonstrated structural alterations in PET microplastics and impacted human gut microbiota communities [26,27]. However, the role of PET hydrolytic enzymes in the gut microbiota in this process has not been well elucidated to date. A recent study revealed the existence of PET hydrolase in human saliva metagenome [28]. Considering the transient exposure of plastic particles in the oral cavity, the degradation process of plastic particles is more likely to occur in the gastrointestinal environment.

In this study, we implemented a bioinformatics workflow to predict PET hydrolase candidates from the human gut metagenomic database [29]. Five promising PET hydrolase candidates, named HGMPs (HMGP01–05), were selected for further biochemical analysis. Among all the HGMPs, HGMP01 exhibited a considerable enzymatic activity for the hydrolysis of PET MPs across diverse temperatures and pH conditions. Further analysis delved into the species and sequence characteristics of HGMP01 and its homologous genes. HGMP01-like genes were identified to be widely distributed throughout the human gut microbiome, with over 20 % of them exhibiting varying levels of expression according to metatranscriptome analysis. These findings shed light on the PET hydrolysis capability of the human gut microbiota, revealing a largely underexplored impact of plastic ingestion on human health.

2. Materials and methods

2.1. Metagenomic data mining

The database of the Human Gastrointestinal Genome was derived from the project of UHGG [29] (http://ftp.ebi.ac.uk/pub/databases/metagenomics/mgnify_genomes/human-gut/v1.0/), and the Unified Human Gastrointestinal Protein catalog (UHGP-90) served as the repository for the putative PET hydrolases. The amino acid sequence of *Is*PETase was used as the query for BLASTP (Protein-Protein BLAST 2.12.0+) search in UHGP-90 with the e-value cutoff of 1e-1. The resulting PET hydrolase candidates were aligned with *Is*PETase using the MUSCLE algorithm in MEGA11 [30] and filtered with the catalytic triad of *Is*PETase using Python script. Signal peptides were predicted by SignalP 6.0 [31]. DLH domain (DLH.hmm) served as the Hidden Markov Model for hmmsearch (HMMER 3.3.2) [32] in the filtered sequences with the default options.

The phylogenetic tree was built using MEGA11 and visualized by Interactive Tree Of Life (iTOL v6, https://itol.embl.de/). Protein structures were predicted by ColabFold [33] using the AlphaFold algorithm and displayed by PyMOL version 2.5.2 (https://pymol.org/2/). For species distribution analysis, the UHGP-100 database was employed to search for the homologous sequences of HGMP01 with BLASTP. The obtained homologous sequences were then used to generate a Sequence Similarity Network (SSN) *via* EFI-EST (https://efi.igb.illinois.edu/efi-est/). Then, the SSN was visualized by Cytoscape (version 3.9.1).

2.2. Strains, plasmids, and reagents

Bacterial strains, plasmids and primers used in this study are listed in Table S1 and Table S2. Antibiotics, lysozyme, and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Solarbio (Beijing, China). Amorphous PET film (GfPET, ES301445) was obtained from Goodfellow (Huntingdon, England). Terephthalic acid (TPA), bis (2-hydroxyethyl) terephthalic acid (BHET), 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) were purchased from Aladdin (Shanghai, China). Mono (2-hydroxyethyl) terephthalic acid (MHET) was kindly provided by Professor Rey-Ting Guo from Hubei University. PrimeSTAR Max DNA Polymerase was bought from Takara (Dalian, China). ClonExpress II One Step Cloning Kit (C112) and ClonExpress MultiS One Step Cloning Kit (C113) were purchased from Vazyme (Nanjing, China). Ni–NTA Sefinose Resin was bought from Sangon (Shanghai, China), and PD-10 Desalting Columns were from GE Healthcare (Milwaukee, USA). Oligonucleotides and codon-optimized HGMPs encoding genes were synthesized by BGI (Shenzhen, China).

2.3. Molecular cloning and heterologous expression

The commercially synthesized HGMPs and *Is*PETase encoding genes, with the signal peptides truncated, were cloned into pET32a-LIC downstream of the TEV protease cleavage site, enabling fusion expression of target proteins with an *N*-terminal Trx tag. All plasmids were constructed by seamless cloning using ClonExpress II One Step Cloning Kit. The constructs were subsequently transformed into chemically competent *Escherichia coli* Rosetta-gami 2 (DE3) for protein expression. Lysogeny broth (LB) media supplemented with 25 μg/mL of ampicillin (Amp) and 5 μg/mL of tetracycline (Tc) (LB-Amp/Tc) were used for the following cultures and protein expression. Cells were cultured at 37 °C in 2 L of LB-Amp/Tc until the optical density at 600 nm (OD_{600}) reached ~0.6. The expression was then induced with 0.2 mM of IPTG for 20 h at 18 °C.

2.4. Protein purification

All the following procedures were carried out at 4 °C. Recombinant *E. coli* cells were collected by centrifugation at 6500 ×*g* for 15 min and then resuspended in 40 mL of lysis buffer. Resuspended cells were disrupted by a Scientz-IID ultrasonic homogenizer (Xinzhi, Ningbo, China) for 30 min (285 W, 5 s work time and 10 s interval). Cell debris was removed by centrifugation at 13,000 ×*g* for 1 h. The supernatant was then applied to a column pre-loaded with 5 mL of Ni-NTA Sefinose Resin. After washing with two times of 30 mL of lysis buffer and 30 mL of wash buffer, the target protein was eluted with elution buffer. The buffer for the collected fractions was then exchanged for 50 mM Tris buffer (pH 7.5) containing 150 mM of NaCl by PD-10 desalting columns. The resulting protein fraction was then digested with 50 μg/mL of His_6-tagged tobacco etch virus (TEV) protease overnight to remove the *N*-terminal Trx tag. The tag-free target proteins were obtained by a second round Ni-NTA affinity by collecting the flow-through fractions. Protein concentrations were determined by monitoring the absorbance at 280 nm on a Nano-300 microvolume spectrophotometer (Allsheng, Hangzhou, China). The purified proteins were aliquoted, flash frozen by liquid nitrogen, and stored at −80 °C until later use for enzymatic assays. The purity of each protein was determined by SDS-PAGE analysis. All buffers used during the purification procedure are listed in Table S3.

2.5. Preparation of PET nano particles

PET nano plastics were prepared from amorphous GfPET film as previously described [34] with appropriate modifications. Briefly, 2 g of GfPET films were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol to a final volume of 100 mL and filled into a burette. This solution was dropped into 100 mL icy double distilled water at a volume flow rate of 1 mL/min through a magnetic stirrer at 2000 rpm. The precipitated polymer was filtered off with a pleated filter (5971/2 FOLDING FILTER). The organic solvent was then removed from the mixture using a rotary

evaporator. After dewatering, the nanoparticles were weighted, and the final concentrations of PET nanoparticles were calculated as 0.5 g/L. PET nanoparticle sizes were determined by scanning electron microscope (SEM).

2.6. Enzymatic assays

The PET hydrolytic activity towards PET nanoparticles was measured *in vitro*. Briefly, purified enzymes were incubated with 0.5 mg/mL PET nanoparticles in 200 μL of 50 mM glycine-NaOH (pH 9.0–11.0) or 50 mM sodium phosphate (pH 6.0–8.0) buffer at a series of temperatures (25–45 °C). The reaction mixture was incubated for 24 h, after which PET nanoparticles were removed by centrifugation at 14,000 ×*g* for 10 min. The enzymes were subsequently denatured by heating at 100 °C for 10 min and removed by centrifugation at 14,000 ×*g* for 10 min. The supernatants were analyzed by HPLC for detection of PET hydrolysis products.

2.7. HPLC analysis of PET hydrolysis products

HPLC analysis was carried out on an Agilent 1220 system, equipped with a pump module, an autosampler, a column oven constantly at 30 °C, a UV detector and a ZORBAX SB-C18 column (4.6 × 250 mm, 5 μm, Agilent, Santa Clara, CA, USA). Mobile phase A was water containing 0.1 % trifluoroacetic acid (TFA), and mobile phase B was methanol containing 0.1 % TFA. TPA, MHET and BHET were separated by the mobile phase as follows: 0–5 min, 15 % B; 5–20 min, 15 %–100 % B in gradient; 20–25 min, 100 %–15 % B in gradient; 25–30 min, 15 % B. The flow rate was fixed at 1 mL/min and the detection wavelength was 254 nm. The concentrations of TPA, MHET and BHET were calculated according to the standard curves of authentic samples of each compound.

2.8. Transcriptomic analysis of HGMP01 homologous genes

The homologous gene expression of HGMP01 was confirmed through the following steps: 1) Metadata was retrieved from the SRA run selector; 2) Raw sequencing data was acquired using fasterq-dump from the SRA toolkit (version 2.10.9); 3) Manually curated HGMP01 homologous genes were given as input to salmon index; 4) Raw RNAseq data were then quantified against the manually curated gene sequence database using the salmon quant command with the validateMappings option for better performance. The raw counts of reads mapped per gene were normalized to TPM values for downstream analysis. The results were then processed with custom shell and python scripts and visualized using the GraphPad Prism (version 9.0.0).

3. Results

3.1. Mining PET hydrolase candidates in the human gut metagenome

The prevalence of PET hydrolytic enzymes has been demonstrated in various ecosystems, including continental environment [35], marine environment [36], and even the human oral cavity [28]. However, given a complex microbial ecosystem and abundant protein resources, the biochemical evidence for PET degradation in the intricate human intestine environment remains unexplored. With the motivation to identify active PET hydrolases in the human gut metagenome, we explored the Unified Human Gastrointestinal Genome (UHGG) collection [29], a public database consisting of 4644 gut prokaryotic genomes and high-quality metagenome-assembled genomes (MAGs). The Unified Human Gastrointestinal Protein (UHGP) catalog associated with the UHGP-100 catalog contains >170 million protein sequences clustered at 100 % amino acid identity; the UHGP-90 catalog contains >10 million protein sequences clustered at 90 % amino acid identity.

In our pursuit of discovering PET hydrolases from the UHGP catalog, we initiated a systematic bioinformatic workflow (Fig. 1a). This process began with a BLASTP search of the UHGP-90 using *Is*PETase [25] as the query, which yielded 172 amino acid sequences. Subsequently, we performed multiple sequence alignment of the 172 amino acid sequences using the MUSCLE [37] algorithm in MEGA11 [30], and 18 sequences were selected as they contained the required catalytic triad residues (*e.g.*, S160-D206-H237 in *Is*PETase). Further analysis using SignalP 6.0 [31] prediction identified 11 sequences containing signal peptides, which is crucial for the extracellular hydrolytic degradation of polyester substrates. We also noticed that among the 80 reported PET hydrolytic enzymes (Table S4), 42 of them contain the DLH conservative domain. This insight led us to perform an hmmsearch [32] on the 11 sequences using the DLH domain (Pfam ID: PF01738) as the model. A total of five putative PET hydrolases were eventually selected for further characterization, including HGMP01, HGMP04 and HGMP05 featuring the DLH domain, while HGMP02 and HGMP03 lack the DLH domain. The HGMPs' sequences are shown in Table S5. The basic properties of these proteins were predicted using Expasy (https://web.expasy.org/protparam/), and the results along with the annotated species information are displayed in Table 1.

3.2. Bioinformatics analysis of HGMPs

To gain a deeper understanding of the relationship between the five candidates and the 80 known PET hydrolases, we crafted a sequence similarities heatmap by TBtools-II [38] (Fig. S3) and then constructed a phylogenetic tree (Fig. 1b) with MEGA11 software [30]. This analysis encompasses a spectrum of high-activity natural PET hydrolases, including *Is*PETase [25], LCC [35], TfH [39] and PE-H [40]. The HGMPs exhibited sequence similarities ranging from 2 % ~ 41 % to previously characterized PET hydrolases (Table S6).

Phylogenetic analysis (Fig. 1b) depicted three distinct branches grouping PET hydrolases from various origins. Except for PES—H1, PES—H2, PHL-1, PHL-2, PHL-4, PHL-5, PHL-6 and LCC which are sourced from uncultured metagenomes, all other enzymes in the yellow branch are derived from Gram-positive bacteria. However, Enzyme504 in the yellow branch is the only exception as it belongs to Gram-negative bacteria. The pink branch exclusively harbors enzymes from Gram-negative bacteria, with a subset retrieved from metagenomes. Intriguingly, the blue branch, housing all HGMPs, accommodates enzymes from both Gram-negative and Gram-positive bacteria, along with contributions from fungi and archaea. Meanwhile, we predicted the structure of HGMPs (Fig. 1c) using ColabFold [33], and the outcomes revealed distinct surface charges for each candidate enzyme, encompassing both the global and local active centers.

3.3. PET hydrolytic activity of HGMPs

We initially assessed the PET hydrolysis activity of HGMPs under the reaction conditions mimicking the preferred pH 9 for *Is*PETase and other PET hydrolytic enzymes [41], along with the human body temperature of 37 °C. Consistent with reported PET hydrolases [25,42], BHET, MHET and TPA were identified as detectable hydrolysis products and quantified using HPLC (Fig. 2a). In our experimental setup, the cumulative quantity of these three compounds was considered as the PET hydrolysis products resulted from the catalysis of the purified enzymes (Fig. 2b). Given that plastics predominantly appear in the human gastrointestinal tract as microplastics (MPs) or smaller particles, we employed nanoplastics derived from amorphous PET film as the reaction substrates for PET hydrolysis evaluation (Fig. 2c). Notably, HGMP01 exhibited remarkable PET hydrolysis activity, surpassing all other candidates (Fig. 2d). Consequently, we narrowed our focus to investigate the optimal conditions for HGMP01. Therefore, we conducted experiments on HGMP01 under varying temperature, pH, and enzyme concentration gradients using PET nanoparticles as substrate.

The temperature gradient experiment determined the optimal

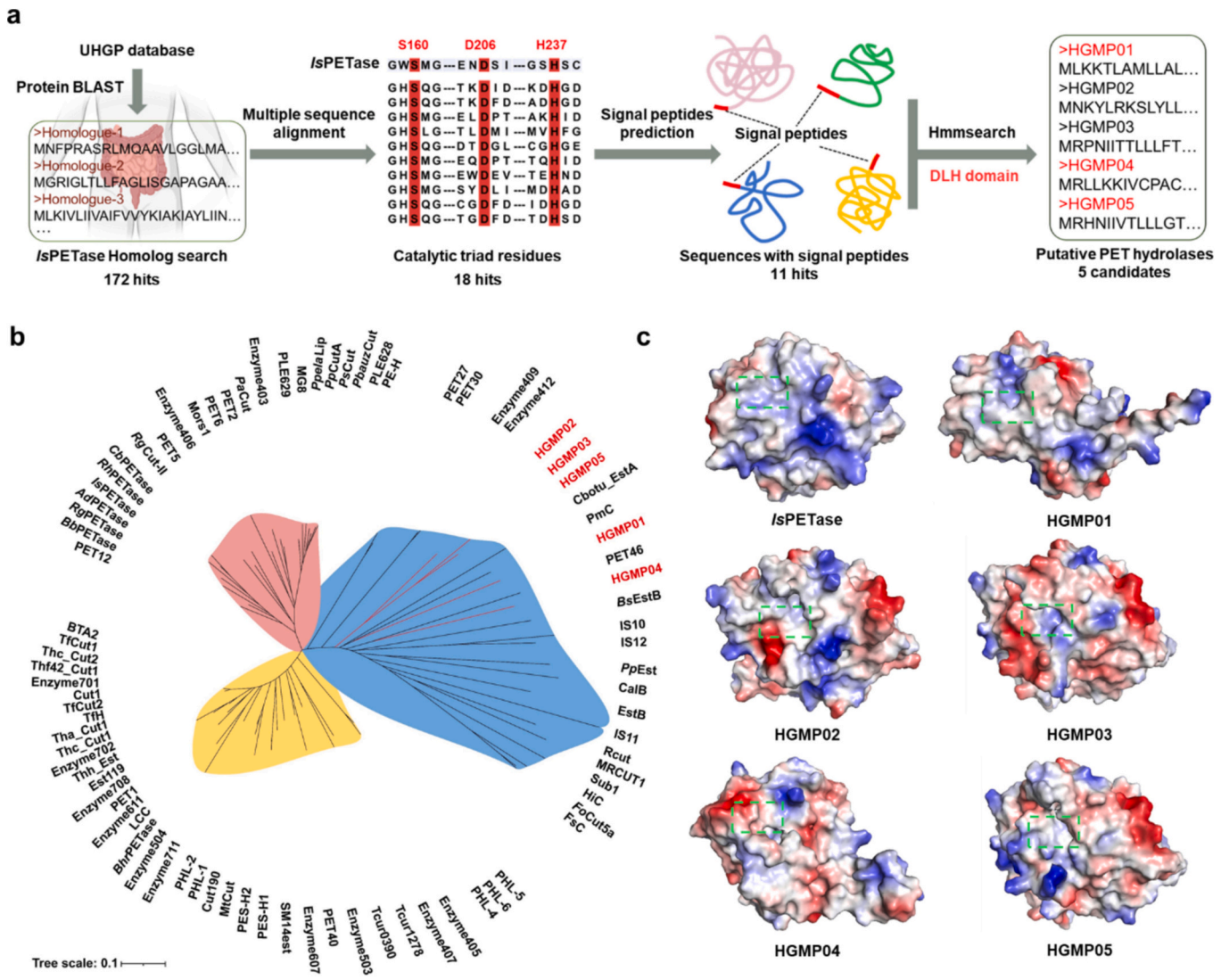

Fig. 1. Identification of putative PET hydrolytic enzymes from the human gut metagenome. a, Bioinformatic work workflow. *Is*PETase homologues were filtered based on the presence of catalytic triad residues and signal peptide. An HMMER search using DLH domain was further performed on the resulting 11 sequences, and five candidates were selected for experimental validations. b, Sequence-based classification of putative PET hydrolases. The neighbor-joining phylogenetic tree of putative PET hydrolases and known PET hydrolases was built using MEGA11, and visualized using iTOL. c, Electrostatic potential maps of modelled structures of HGMPs. The structure of *Is*PETase was from PDB database (PDB ID: 5XG0), and HGMP structures were predicted using ColabFold. All structures were visualized by PyMOL version 2.5.2 (https://pymol.org/2/). Active site of each protein is enveloped within a green dashed box. Red and blue colors represent negative (acidic residues) and positive (basic residues) potentials, respectively (scale of −100 to +100 k_BT/e_c).

Table 1
Information and protein properties of the selected HGMPs from the human gut metagenome.

Protein ID	Sequence length	Molecular weight (kDa)	Predicted pI	Presence of DLH domain	Host taxonomy[a]
HGMP01	275	30.18	9.12	yes	Clostridia: Acutalibacteraceae
HGMP02	341	38.17	5.37	no	Bacteroidia: Porphyromonadaceae
HGMP03	323	36.29	6.09	no	Bacteroidia: Porphyromonadaceae
HGMP04	282	30.65	6.50	yes	Alphaproteobacteria: Rhizobiaceae
HGMP05	321	36.24	8.30	yes	Bacteroidia: Porphyromonadaceae

[a] The taxonomy of protein hosts is indicated as Class: Family based on the annotation of the UHGG database [29].

activity temperature for HGMP01 as 40 °C (Fig. 2e), with sustained high activity at the human body's optimum temperature of 37 °C. Furthermore, we determined the optimal pH for HGMP01 to hydrolyze PET nanoparticles. As is shown in Fig. 2f, HGMP01 exhibited broad tolerance in the pH range of 7.0–11.0, with heightened activity under mildly alkaline conditions. The enzyme displayed its maximum activity at pH 10.0–11.0. Consistent with our previous speculation, an increase in pH promoted the hydrolysis of PET nanoparticles. Moreover, a proportional increase in PET hydrolysis products was observed with the ascending concentration of HGMP01 from 50 to 2000 nM (Fig. 2g). This result contrasts with *Is*PETase when PET film was employed, which attained peak product concentrations with the addition of 200 nM enzyme [43]. This outcome suggests increased potential for the degradation of small PET particles with higher enzyme concentrations.

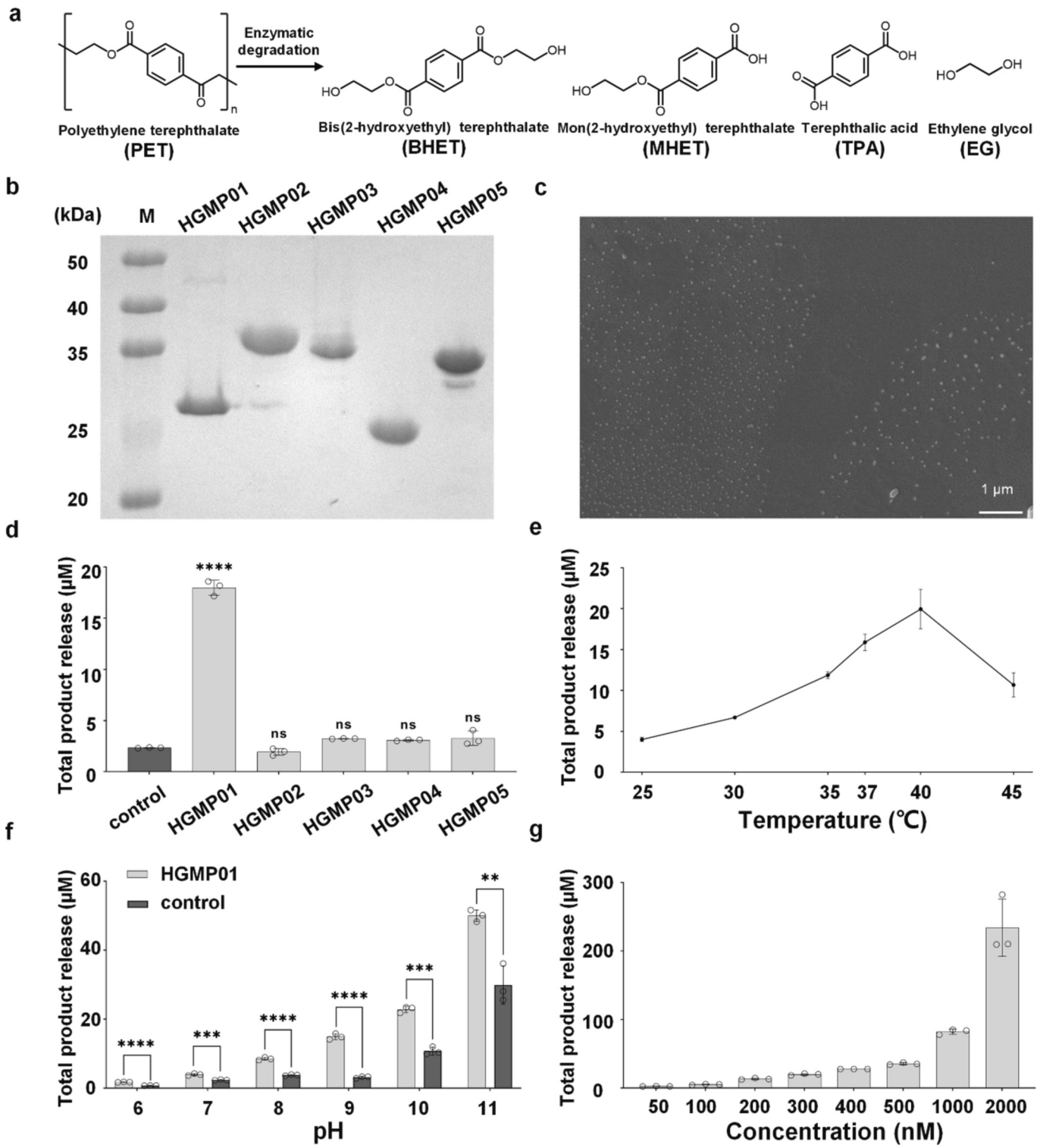

Fig. 2. Purification of HGMPs and biochemical characterization of HGMP01. a, products of enzymatic hydrolysis of PET. b, SDS-PAGE (15 %) analysis of HGMPs. The sizes of the molecular weight standards (M) are indicated on the left. c, SEM image of PET nanoplastics. d, PET hydrolytic activity of HGMPs. The reaction temperature for each group of enzymes were 37 °C. Glycine-NaOH (pH 9.0) buffer was used for all enzymes. Statistical analysis was conducted using one-way ANOVA to compare the release of PET monomers between the enzyme-free control and the HGMPs. $^{****}P < 0.0001$; ns, no significance. e, HGMP01 catalyzed PET hydrolysis under a temperature gradient of 25, 30, 35, 37, 40 and 45 °C. f, HGMP01 catalyzed PET hydrolysis under a series of pH conditions. Phosphate buffer was employed for generating the pH range of 6.0–8.0, and Glycine-NaOH buffer was applied for the pH range of 9.0–11.0. Statistical analysis was performed using multiple unpaired *t*-tests to compare the release of PET monomers between the enzyme-free control and the HGMP01 catalyzing group across different pH conditions. $^{****}P < 0.0001$; $^{***}P < 0.001$; $^{**}P < 0.01$. g, PET hydrolysis products released under a series of HGMP01 concentrations. All measurements were conducted in triplicate ($n = 3$).

3.4. Distribution and diversity of HGMP01-like enzymes in the human gut microbiome

We next delved into the distribution of HGMP01-like enzymes within the human gastrointestinal microbiome. Specifically, HGMP01 sequence served as the query for homologue search in the UHGP-100 database. As is shown in Fig. 3a, homologue search identified a total of 697 putative HGMP01-like enzymes (amino acid sequence identity ≥20 %, e-value ≤1e-3). These enzymes were distributed across 41 families and 94 genera of microbial species, including some unclassified species, suggesting the widespread occurrence of HGMP01-like enzymes in human gut microbiota. The majority of the HGMP01-like enzymes are prevalent in *Firmicutes* bacteria, a predominant group in the gut microbiota, in which *Dialisteraceae* family occupies the largest proportion. Additionally, HGMP01-like enzymes were also found in *Actinobacteriota*, *Bacteroidota*, *Proteobacteria*, and even in the domain of Archaea, with some of these bacterial species being pathogens typically colonized in the digestive tract.

To gain deeper insights into their relationship, we conducted a sequence similarity network (SSN) of aforementioned 697 HGMP01-like proteins along with HGMPs. As is shown in Fig. 3b, HGMP01 and seven homologues form a unique cluster, which is distinct from the other four HGMPs. Notably, the proteins clustered with HGMP01 are all from the *Acutalibacteraceae* family, suggesting that this family may be a key contributor to the intestinal function of PET hydrolysis.

3.5. Transcriptomic profile of HGMP01 homologous genes in PET hydrolysis

Furthermore, we sought to confirm the activated transcription of HGMP01 and its homologues alongside the existing genomic evidence. Leveraging metatranscriptomic data from the Health Professionals Follow-up Study (HPFS) [44], a transcriptional analysis was conducted and refined based on the insights gained from the SSN analysis, emphasizing HGMP01's unique classification. This targeted approach led to the selection of specific homologous proteins of HGMP01 for transcriptome analysis (Fig. 4a). By examining over 100 homologous genes expressed in the HPFS data samples, we identified eight genes

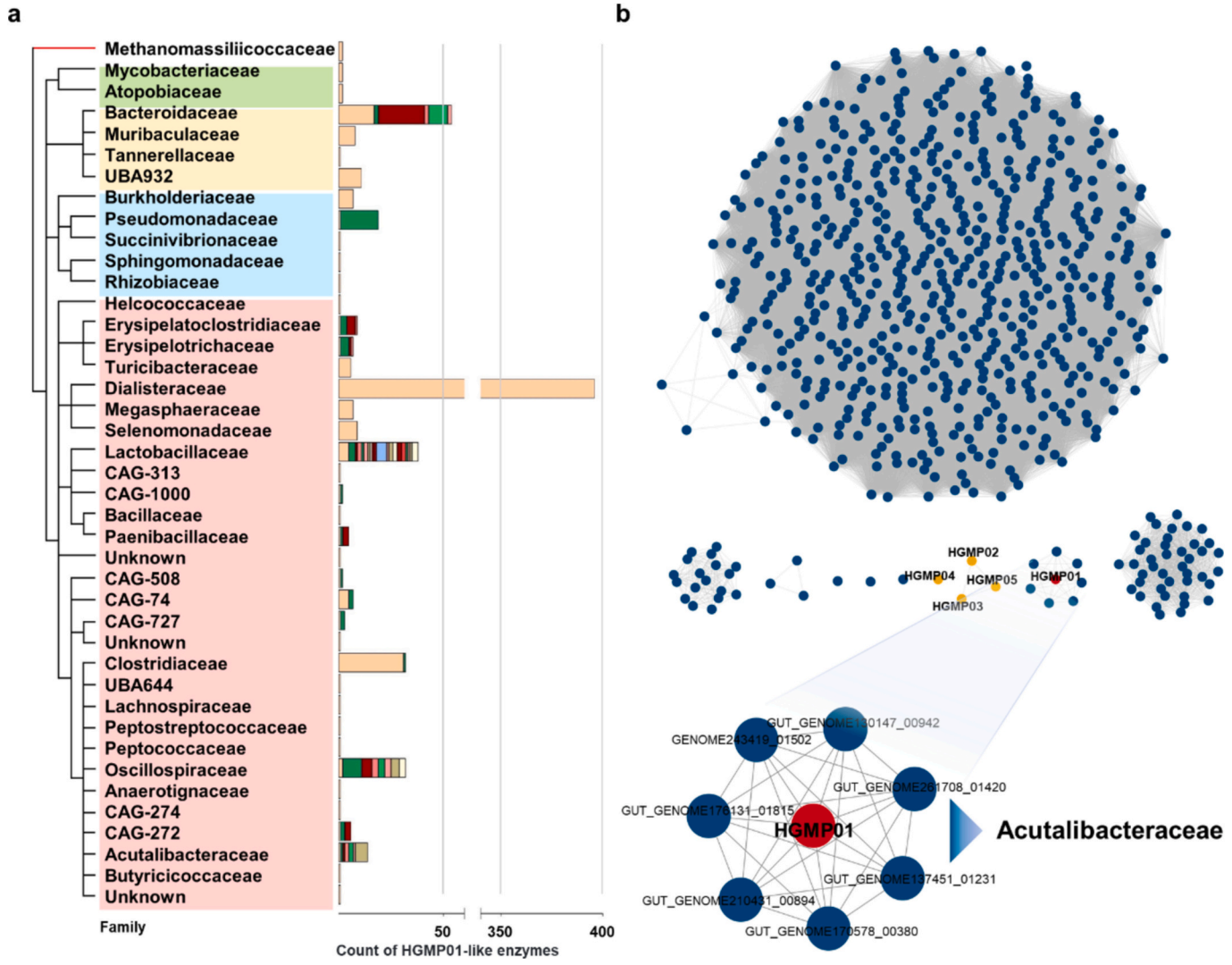

Fig. 3. Species distribution and similarity clustering of HGMP01-like enzymes in human gut microbiota. a, Species distribution of HGMP01-like enzymes. A phylogenetic tree was constructed based on the information form 41 families provided by UHGG in Taxonomy CommonTree and visualized using iTOL. The red branch on the phylogenetic tree represents archaea, while the rest represent bacteria. Bars in the right panel represent the number of HGMP01-like enzymes distributed in each family, with colour differences indicating various genera. b, Sequence similarity network (SSN) of HGMP01-like enzymes. The cluster containing HGMP01 was highlighted in red, and the cluster containing HGMP02, HGMP03, HGMP04 and HGMP05 was highlighted in yellow. Datasets were generated through EFI-EST and visualized using the Cytoscape software. SSN was built with an e-value threshold of 5, minimum sequence length of 150 residues, and an alignment score of 40.

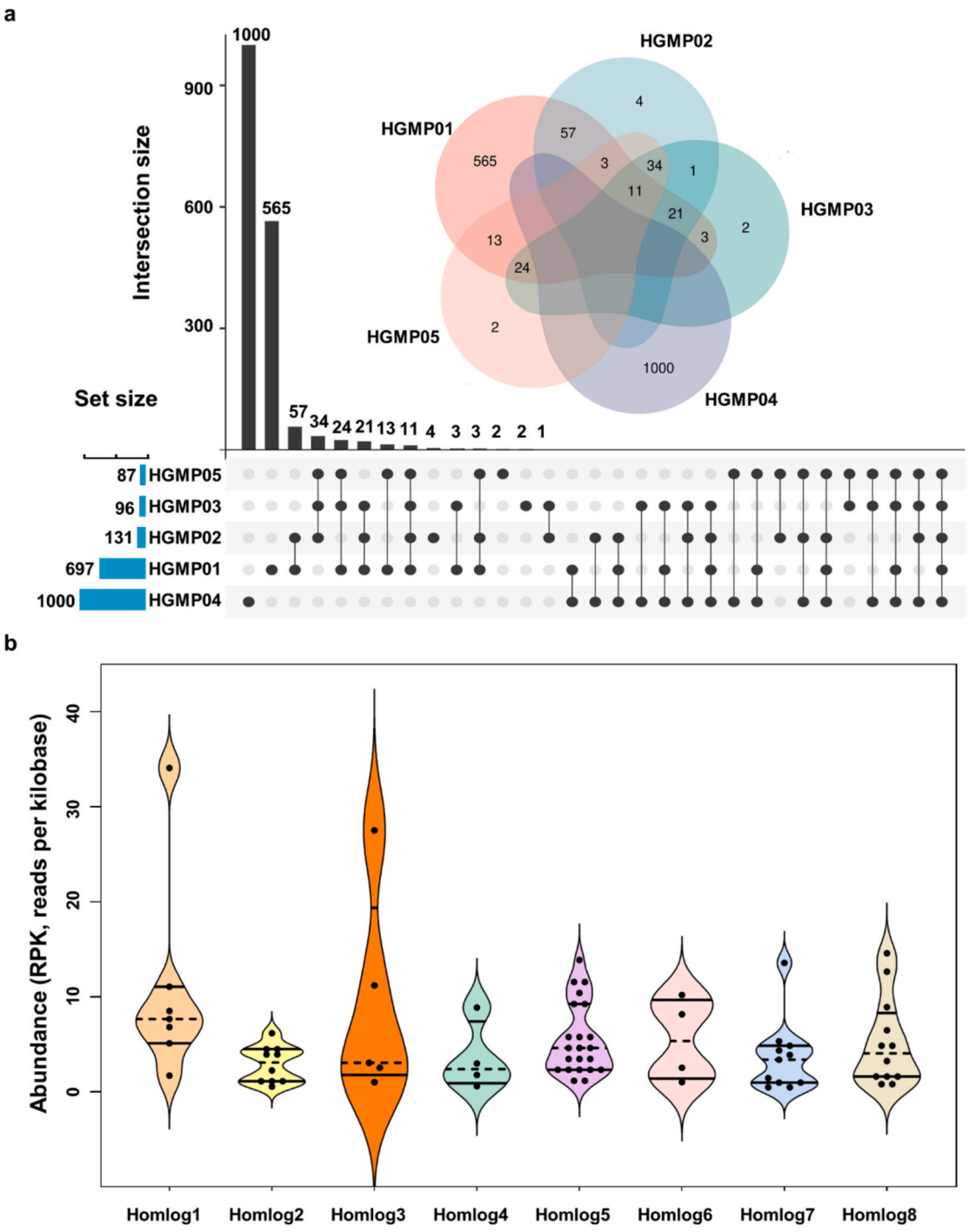

Fig. 4. Expression profile of unique homologous proteins of HGMP01. a, Identification of unique homologous proteins of HGMP01. Five categories of HGMP-like enzymes were depicted in a Venn diagram, and a total of 565 HGMP01 unique homologues were identified for subsequent transcriptome analysis. b, Expression levels of HGMP01-like enzymes. Metatranscriptomic reads from HPFS (number of samples = 378) were aligned to the gene sequences identified in Fig. 4a using Salmon and visualized by the GraphPad. The top eight homologues with the highest expression levels are displayed. Detailed sequence information of Homolog1–8 is available in Table S7.

with relatively higher expression levels across multiple samples, providing a nuanced view of the transcriptional dynamics (Fig. 4b). This comprehensive analysis provides a nuanced understanding of the intricate relationships and gene expression patterns of HGMP01 and its homologues, particularly in the context of PET hydrolysis within the human gastrointestinal microbiome.

4. Discussion

Plastics have been produced and widely used on a large scale over the last 70 years [45,46]. While the evolution time of microorganisms or enzymes for plastics degradation is very limited, various PET hydrolytic enzymes have been identified in nature, including carboxylesterases, lipases, and cutinases [47,48]. In this study, the discovery of PET-degrading enzymes from the human gut metagenome demonstrates an unprecedented function of intestinal microbiota. Sequence alignment

analysis of known PET hydrolases (Fig. S3) revealed that HGMPs exhibited relatively low sequence similarity to other PET-degrading enzymes.

In the PET degradation process, the formation of anionic carboxyl group led to the gradual increase of negative charge on PET surface, which attracts PET hydrolases harboring positive charges on the surface [49]. Therefore, the enhancement of positive charges on the enzyme surface can improve PET degradation in principle [50]. Predicted electrostatic potential mapping based on protein structure models (Fig. 1c) suggests that HGMP01 and HGMP05 enzymes exhibit overall positive charges on the surface. Conversely, other candidates display neutral or negative surface charges, consistent with their predicted isoelectric points (Table 1). An overall pI of 9.12 and a near-neutral active center was predicted for HGMP01, implying that negative surface charges, combined with a near-neutral active center, may have a positive contribute to the PET hydrolysis activity.

The intestines are densely colonized by thousands of microbial species and constitute the largest micro-ecosystem of human body [51,52]. The gut microbiome harbors over 3.3 million genes, approximately 100 times that of the human genome. The gut microbiome, alongside the human genome interacts with environmental factors to exert diverse effects on human health. Intestinal microbes play a pivotal role in the digestion and metabolism of indigestible carbohydrate such as xylan, cellulose, and resistant starch [53–55]. These microbial activities result in breakdown of polysaccharides into monosaccharides and short-chain fatty acids, enhancing food utilization efficiency of the host. Previous studies have linked MPs in the human intestine to gastrointestinal diseases such as inflammatory bowel disease (IBD) [10]. Herein, the identification of HGMP01 revealed that the gut microbiota should also exhibit the capability to hydrolyze PET, which was substantiated by *in vitro* functional validation. This discovery broadens the functional repertoire of intestinal microorganisms, challenging the conventional notion that humans cannot digest and utilize plastics. Additionally, TPA and EG represent the primary hydrolysis products of PET. An *in vitro* study indicates that TPA and EG have no adverse effects on human fibroblast cells and *Caenorhabditis elegans* under conditions below 200 μg/mL [56]. Hence, the presence of PET hydrolases in intestinal and saliva microorganisms holds immense significance to human health amid the pervasive prevalence of plastics.

The evolution of PET hydrolases in gut microbes appeared to have occurred independently, as indicated by their distinct distribution in gut microbiomes (Fig. 4a). The most homologous sequences of HGMP01 were distributed in the family of *Dialisteraceae*, *Bacteroidaceae*, *Lactobacillaceae* and *Oscillospiraceae*, spanning across multiple genera of *Lactobacillaceae*, *Oscillospiraceae* and *Acutalibacteraceae*. Notably, *Bacteroidaceae*, a predominant Gram-negative bacterium in the human gut, thrives as a strictly anaerobic species, establishing a long-term, mutualistic relationship within the host intestine. Recognized for its richness in polysaccharide utilization loci, *Bacteroidaceae* encodes numerous glycoside hydrolases, conferring a remarkable capacity for polysaccharide degradation [57]. Similarly, *Oscillospiraceae* and *Acutalibacteraceae* were reportedly proficient in fermenting complex plant carbohydrates [58,59], which provides conducive conditions for the evolution of PET hydrolysis activities.

While this manuscript was in preparation, another group independently discovered enzymes capable of degrading PET in cultivable gut microbiota [60]. However, HGMP01 identified in this study from the human gut microbiome shares only 5.9–23.4 % sequence identity with those enzymes. Furthermore, the wide distribution and varying levels of expression of HGMP01-like genes was recognized in this study through global metagenome and metatranscriptome analysis. Taken together, these findings suggest that PET hydrolytic enzymes may be more widely distributed in the human gut microbiome than ever estimated.

5. Conclusion

In summary, we systematically delved into UHGG metagenome data, leading to the identification of a novel PET hydrolase, HGMP01, within the human gastrointestinal metagenome. The PET hydrolysis capability of the enzyme was verified through *in vitro* biochemical characterizations. Evolutionary analysis suggests that this enzyme may have originated independently. Based on the distribution of bacterial strains and transcriptomic analysis, we posit that the humans gut microbiota exhibits the ability to degrade PET particles. Our findings not only provide enzymatic evidence supporting the capacity of human intestinal microorganisms to break down PET plastics, but also underscore the potential mitigation of the adverse health effects associated with MPs.

To date, over 100 PET hydrolyzing enzymes have been identified across diverse natural environments [61], including recently discovered highly active variants from deep-sea habitats [62], highlighting the extensive distribution of such enzymes in the biosphere. Our study contributes to this field by demonstrating, from a specific perspective, that human gut microbiota also possesses the capacity to produce PET hydrolyzing enzymes. However, given the relatively short transit time of microplastics within the human gastrointestinal tract, *in vitro* data alone may not sufficiently reflect the magnitude of the gut microbes' ability for clearing exogenous PET microplastics. A comprehensive assessment of this capacity may require targeted human clinical trials that thoroughly investigate the persistence and potential breakdown of PET microplastics within the human gastrointestinal system, as well as any associated health effects arising from microbial interactions or soluble compounds released during the degradation process.

CRediT authorship contribution statement

Guoqiang Zhang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jieke Du:** Validation, Investigation. **Chengsong Zhang:** Validation, Investigation. **Zhiyi Zhao:** Validation. **Yuexing Chen:** Validation. **Mingyu Liu:** Validation. **Jianwei Chen:** Investigation. **Guangyi Fan:** Investigation. **Li Ma:** Writing – review & editing, Investigation, Funding acquisition. **Shengying Li:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Kun Liu:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

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.ijbiomac.2024.137732.

Data availability

The data supporting the findings of this study have been deposited in Science Data Bank (DOI:10.57760/sciencedb.15087).

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