Microbes Isolated from Landfill Soil Utilize Polyethylene Terephthalate (PET) as Their Sole Source of Carbon: An Unexplored Possibility of Bioremediation in Bangladesh

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ABSTRACT

Plastic products are so extensively used that they continue to strain the already overburdened waste management system and, inevitably, the global climate. Biodegradation is a sustainable remedy. Here, we report a few microorganisms isolated from landfill soil near Dhaka that thrive especially on polyethylene terephthalate (PET) polymers. Soil samples were subjected to three enrichment cycles that contained no carbon except PET. Pure isolates were recovered and incubated on minimal agar containing PET as the sole carbon. A morphological examination was carried out. Potential PET-degrading enzyme sequences from the isolates and other microalgae were analyzed for homology using BLASTP and TBLASTN, and multiple sequence alignment (MSA) was performed to assess conserved domains. Six isolates were obtained. Two isolates grew around the PET film but did not grow sufficiently in other areas of the minimal agar. Two other isolates with greenish pigmentation flourished around the PET film as well as on other areas of the agar. One of the green cells resembled *Aphanocapsa*, with irregular shapes and occasionally brown dense bodies, while the others looked round like *Microcystis*. Homology analysis revealed the hypothetical PETases in green cells contained the highly conserved catalytic triad (Ser-His-Asp) at the active site, as always found in alpha-beta hydrolase fold containing enzymes. Microbes isolated from two landfill sites in the vicinity of Dhaka have been adapted to utilize PET as a carbon source. In the future, sequencing and further characterization would be necessary to validate the findings. Microalgal systems demand increased focus, given their potential to offer valuable resources for bioremediation.

1. INTRODUCTION

Polyethylene terephthalate (PET or PETE) is one of the most used types of plastic for many attractive features. Aromatic terephthalic acid and ethylene glycol give rise to this linear polymer with excellent mechanical and thermal properties. Oftentimes, we use the single-use versions of it for its appealing qualities. However, the same appears as a curse as piled-up plastics in the natural ecosystem continuously pose a threat to our earth, including clogging issues, habitat ruining, animal entrapment, and microplastic-mediated toxicity to the nervous and reproductive system (Barnes et al., 2009; Waring et al., 2018). The difficulties in the degradation of PET arise from its molecular weight (Urbanek et al., 2021) high degree of crystallinity, failure to act as substrate, and high Tg (glass transition temperature) (Mohanan et al., 2020; Brott et al., 2022). Part of the ecological burden has been attempted to solve by taking recycling initiatives. Recycling, which may take many forms, is currently practiced mainly through melt extrusion and glycolysis (Park et al., 2014). However, these processes are not cheap or efficient, let alone eco-friendly. Biodegradation is an extremely desirable alternative. Nature has excellent capacity by dint of its collection of microbes to adapt and tweak its existing enzyme pool to create a new variety that can degrade new substrates. Past attempts to find enzymes that can degrade PET provide evidence

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of microorganisms or their enzymes' ability to break down these notoriously resilient synthetic chemicals (Malafatti-Picca et al., 2019; Carniel et al., 2017; Gamerith et al., 2017; Yang et al., 2013). *Ideonella sakaiensis* PETase (IsPETase) isolated from Japan has been one of the top-performing mesophilic PET metabolizing enzymes (Yoshida et al., 2016; Samak et al., 2020; Carr et al., 2020). The enzyme is secreted when the substrate is available after which it breaks down the PET into MHET (mono-2-hydroxyethyl terephthalate), BHET (bis-hydroxyethyl terephthalate-late), TPA (terephthalic acid), and EG (ethylene glycol) along with MHETase. PETases are enzymes similar to cutinase, and they have been demonstrated to possess the ability to biodegrade PET. Similar to cutinase, other hydrolases such as lipase and carboxylesterase have previously been reported to exhibit biodegradation activity on PET (Han et al., 2017). Studies have revealed that these enzymes share a common characteristic: they all contain the typical serine hydrolase fold at their active sites, along with the Ser-His-Asp catalytic triad (Wei et al., 2016; Han et al., 2017; Joo et al., 2018; Austin et al., 2018). Other PET degradation activities with the same catalytic elements have been reported in fungi such as *Fusarium* and *Humicola* cutinases, *Candida antarctica* lipase CalB as well as *Aspergillus* and *Penicillium* sp., etc. (Carniel et al., 2017) and in bacteria such as *Thermobifida* cutinases (Barth et al., 2016; Silva et al., 2011; Wei et al., 2016) and *Saccharomonospora viridis* cutinase type polyesterase (Kawai et al., 2014). Most of the reports on plastic biodegradation in Bangladesh have been on LDPE (Low-Density Polyethylene) (Hossain et al., 2019; Biki et al., 2021) where isolates such as *Ralstonia* sp. strain SKM2 and *Bacillus* sp. strain SM1 were found to exhibit plastic breakdown activity.

Our study aims to isolate strains from the natural microbial community evolving to utilize PET in landfill soil near Dhaka through enrichment culturing. A variety of PET utilization abilities if found in the current study would add to the existing handful list of enzymatic systems. Moreover, it is always desirable to find an alternative option or outperformer in terms of activity and/or other characteristics that are biotechnologically promising such as cheaper feedstock and thermal stability.

2. METHODOLOGY

2.1 Sample collection

Garbage soil samples were collected along with dumped plastic from Matuail and Aminbazar, the two landfills serving the capital city of Bangladesh (Figure 1). The sampling was performed in rounds: At first, in December 2020, and then in April 2022. Matuail, with a 100-acre area located in Demra (south of Dhaka), serves as the city's garbage dump. It is an aged landfill (26 years old) (Akter et al., 2021). Since there is no segregation and recycling facility, with a daily load of 2,500 tons of solid waste from Dhaka's south city corporation areas, it is a place where massive mounds of plastic waste deposition take place (Chandan, 2021). The Aminbazar landfill is l km away from the capital and has been serving mostly the northern part of Dhaka since 2007 (Urme et al., 2021). So, both areas have the potential to facilitate the evolution of enzymes that degrade plastic.

For sampling, we used sterile containers to collect the soil samples at a depth of 9-10 cm. Around 10 g of soil were collected into a sterile Ziplock bag and transported to the laboratory, the temperature was also recorded.

2.2 Preparation of PET strips as the sole source of carbon and for the PET utilization test

The polyethylene terephthalate (PET) sheet (HS Code: 3920.62.90) used in this study was a generous gift from Arbab Poly Pack Ltd. (Bangladesh) (https://www.arbabpolypackltd.com). These PET sheets were 100% Food grade and have been imported from India. PET film was sectioned into 2×3 cm rectangular-shaped pieces of PET strips which were used as the sole source of carbon in the liquid enrichment medium or minimal agar medium. All PET strips were disinfected by autoclaving at 121°C for at least 30 min under 15 psi of pressure. These PET strips were further used in enrichment culturing or performing PET-utilizing tests.

2.3 Inoculum preparation, screening using enrichment medium, pure culture isolation and characterization

One gram of the soil sample along with dumped plastic was dipped into 90 mL NaCl Solution (0.85%) and gently shaken to mix (Jangra et al., 2020). This plastic-associated environment, known as the plastisphere, was used as an enrichment source for plastic utilizing microorganisms (as described by Rüthi et al., 2023). The suspended soil samples were kept for 2 days in a shaker incubator at 120 rpm and at room temperature, and 1 mL of this suspension was used as an inoculum for the enrichment medium (Figure 1).

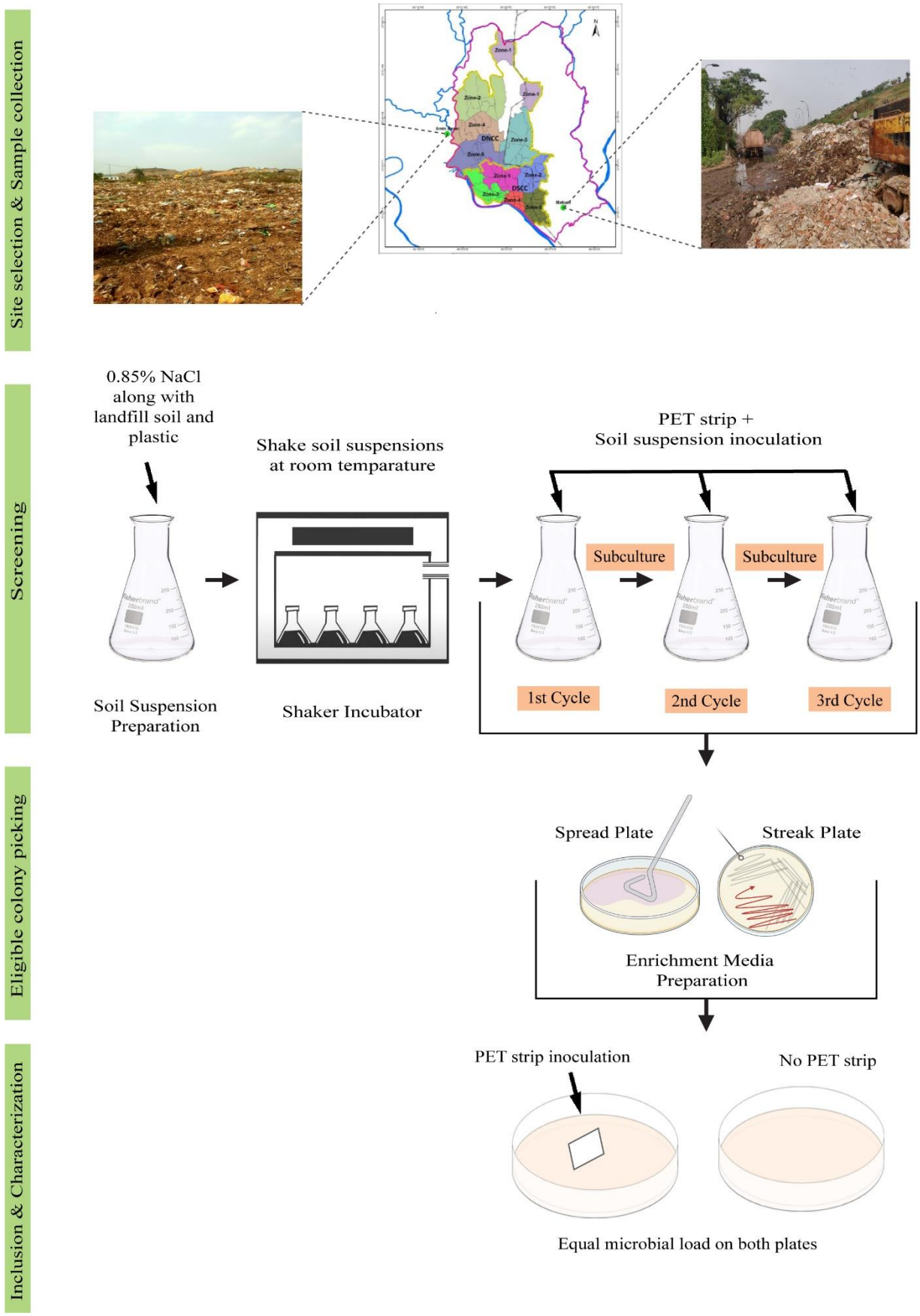


Figure 1. Flow diagram of site selection, sampling, screening, isolation, and characterization of plastic (PET) utilizing microbes from Matuail (left) and Aminbazar (right) landfill soil

For screening, 100 mL of enrichment medium was prepared following Skariyachan et al. (2015) with slight modifications. The enrichment broth contained 1 g/L $NaNO_3$, 0.2 g/L $MgSO_4.7H_2O$, 0.05 g/L $FeCl_3$, 0.02 g/L $CaCl_2$, 1 g/L KH_2PO_4, 1 g/L K_2HPO_4, 1 g/L $(NH_4)_2SO_4$ at pH 7 supplemented with one PET strip (2 × 3 cm rectangular shaped) as the sole source of carbon in a 250 mL Erlenmeyer flask (Figure 1). One mL of the inoculum (prepared soil suspension) was added and then the flasks were incubated for 15 days at ambient temperature in the presence or absence of light (not shown). Negative control was also incorporated into the experiment. It contained the enrichment broth with the PET strip that did not receive any inoculum. After 15 days of incubation, 1 mL of the first enrichment culture was transferred along with the PET strip from the first cycle to a second enrichment broth for another 15 days at ambient temperature. The third round of enrichment also followed in the same way for an additional 15 days. After three cycles of enrichment, 50 µL from both the Matuail and Aminbazar flasks were placed on minimal agar (the same composition as the enrichment broth but supplemented with 1.5% agar) by the spread plate technique. Plates were incubated at 37°C for those that did not receive any light in the enrichment cycles. Another group of plates was incubated at an ambient temperature where there was an ample amount of light. Obtained colonies were isolated by the streak plate method and subcultured repeatedly for pure isolates (Figure 1).

These isolates were further characterized by the PET-utilizing test following the plate test described by Urbanek et al. (2017) with modification. In this study, we designed and performed the PET-utilizing test, by growing individual isolates first in liquid enrichment broth with PET strips as the sole source of carbon and then a hundred microliters of the resulting cultures were dropped and spread on the solid enrichment (minimal) agar plates. The experiment was conducted in parallel using two sets: In one set: an agar plate was overlaid with PET film as the sole organic carbon source while a control plate was set without any carbon source, and both plates were incubated at 37°C (Figure 1). For the second set: two plates were set one with PET films as the carbon source and another without, just like mentioned above, but with different incubation conditions including ambient temperature and sufficient light. After four days of incubation, all plates were checked and growth was compared between the two sets. For both of these sets, a third plate was also incorporated that received no inoculum.

A smear of the isolated pure culture was made on a fresh grease-free slide with a sterile loop. The slide was checked under a microscope to determine the morphology of isolated strains based on shape, size, and color (Sugoro et al., 2022; Najeeb et al., 2022, Badr and Fouad, 2021; Bellinger and Sigee, 2015). Otherwise, a drop of culture was placed under a cover slip and directly observed without stain.

2.4 Glycerol stock preparation

Five hundred microliter overnight cultures of each of the isolates were added in 50% (v/v) glycerol, gently mixed and the tube was frozen at -20°C.

2.5 *In silico* bioinformatics analysis

A homology search utilizing BLASTP and TBLASTN (Basic Local Alignment Search Tool Program and Protein-nucleotide 6-frame translation) was conducted to select potential PETase-like microalgal proteins (performed on 19 October 2022) by selecting the following organisms such as algae (taxid: 3041), green algae (taxid: 3041), red algae (taxid: 2763), blue-green algae (taxid: 1117), yellow-green algae (taxid: 2833), brown algae (taxid: 2870), Shewanella algae (taxid: 38313), *Chlamydomonas reinhardtii* (taxid: 3055), *Dunaliella salina* (taxid: 3046), Chlorella (taxid: 3071), *Botrycoccus braunii* (taxid: 38881), *Phaeodactylum tricornutum* (taxid: 2850), *Thalassiosira pseudonana* (taxid: 35128), *Isochrysis* (taxid: 37098), and *Nannochloropsis* (taxid: 5748). Multiple sequence alignments were conducted using PROMALS3D (http://prodata.swmed.edu/promals3d/promals3d.php) (Pei et al., 2008) on the amino acid sequences of enzymes obtained from the abovementioned searches in addition to previously known PETase enzymes preferably with known structures. The Phyre2.0 fold recognition server (Kelley et al., 2015) was used for protein modeling (normal mode and default parameters). Docking of the substrate against the model proteins was performed using AutoDock Vina (Trott and Olson, 2010) accessible via PyRx (Dallakyan and Olson, 2015).A grid box was specified mainly around the substrate binding and active sites with dimensions (25.0-X, 28.54-Y, 21.45-Z for cyanobacterial enzyme and 25.30-X, 26.05-Y, 23.47-Z for pycnococcal protein) Å and centered at (0.0, -1.77, 2.47 for cyanobacterial protein and -13.24, 30.11, 5.27 for pycnococcal protein) Å. The ligand was docked to the protein with

a default exhaustiveness of 8. The model with the lowest binding energy was chosen. The BIOVIA Discovery Studio Visualizer (Biovia, 2021) was used for model visualization and representation of the intermolecular interactions, and to create a 2D diagram of ligand binding site atoms.

3. RESULTS AND DISCUSSION

3.1 Microorganisms from Aminbazar and Matuail dumpsites showed growth in the enrichment broth utilizing PET film as the sole source of carbon

Enrichment culturing has been previously used for the isolation of microorganisms with novel metabolic capacity (Tortora et al., 2007). The design of the enrichment medium tells which metabolic type is going to be favored. Usually, no selective agent is used in this type of nutritional material; but omission/incorporation of a metabolic condition results in the selection of a specific type of microbe that possesses the desired metabolic trait, and by the same mechanism it does not allow other types of microbes to grow (Tortora et al., 2007).

The enrichment medium used in our study did not contain any organic carbon source except the PET film. Soil samples collected from Aminbazar and Matuail dumpsites were inoculated into the enrichment medium. Enrichment of PET-degrading microbes was carried out in three consecutive 15-day-long subculture screens both in the presence and/ or absence of sunlight. Growth and greenish appearance were noticed at the end of each subculture for cycles that were exposed to light, which were more evident in further subcultures (Figure 2). Quicker growth in the subsequent subcultures and slow response in the first few days after soil samples were introduced in the enrichment medium may represent the lag phase (Rolfe et al., 2012) when the microbes were preparing and acclimatizing with the nutrient and other conditions.

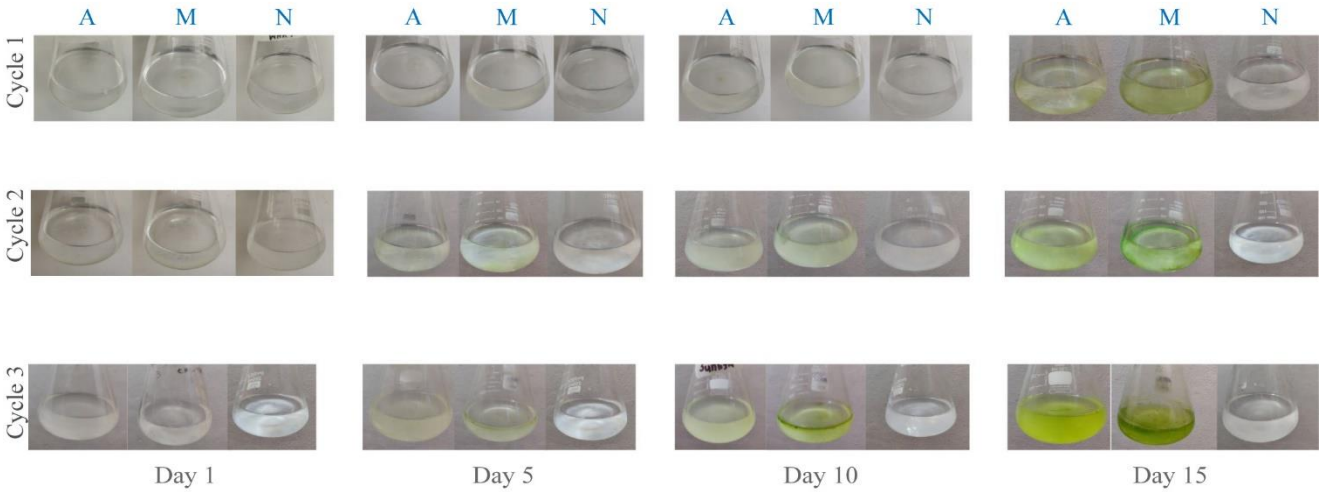


Figure 2. Enrichment analysis of PET utilizing microorganisms in three consecutive 15-day-long subculture stages showing growth and green appearances. 1st cycle: the first 15 days; 2nd cycle: from the 16-30th day; 3rd cycle: 31st- 45th day. M: Matuail; A: Aminbazar; and N=Negative control with PET strip but no inoculum

3.2 PET as the sole organic carbon source for individual isolates on minimal (enrichment) agar plate

Since the liquid enrichment medium contained no other carbon source except plastic film, the growth in the enrichment medium indicates two possibilities: (a) autotrophic growth or (b) growth using PET film as a carbon source. We recovered six pure isolates after repeated streaking and microscopic observations. To test if their growth is the result of either of the two above reasons, equal amounts of the pure cultures were used to make a lawn on two minimal agar plates one of which was laid over with PET films, but the other plate was not (Figure 1).

Both plates were examined on the 4th day of incubation. Two of the isolates showed growth at the edges of the PET film and only scarcely or no growth was observed on the rest of the agar surface. The other four isolates (M2, M4, A1, and A2) were different and showed growth both at the edges of the plastic and all over the agar plates. All isolates, except A2, appeared pleomorphic/irregular, under the microscope while A2 had a round appearance (Figure 3).

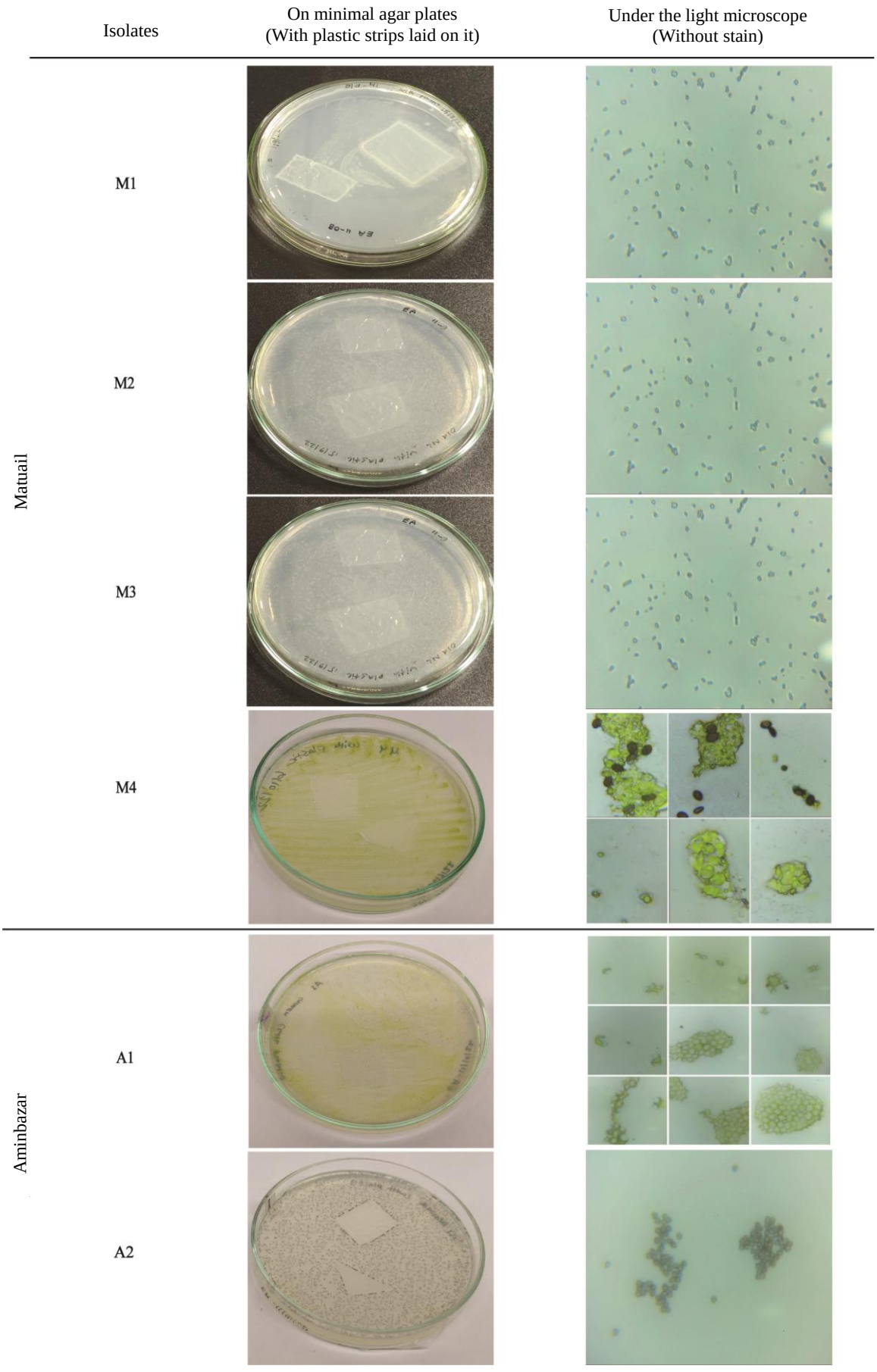


Figure 3. Isolated microbes on minimal agar plates with PET film as the sole organic carbon source and their microscopic observation at 100X (oil immersion) under the light microscope

Apart from the occasional isolated green cells, there were brown dense structures in the M4 micrograph. The irregularly shaped colonial form of M4 resembled amorphous gelatinous colonies of *Aphanocapsa* (Bellinger and Sigee, 2015; Felisberto and Souza, 2014; Gama et al., 2014 and Supplementary Table S1). Although A1 individual cells were sometimes spheroidal, the colonial form of A1 was largely irregular and looked like mucilage-producing *Microcystis* (Bellinger and Sigee, 2015; Pereira and Portillo, 2018; Pham et al., 2021 and Supplementary Table S2). To test if *Aphanocapsa* or *Microcystis* contain any sequence homologous to PETase, we performed BLASTP using *Aphanocapsa* and *Microcystis* as organisms but no homology was found. However, when we searched for esterase sequences in these two types of microbes using simple text mining at the NCBI Protein database, we retrieved three esterases from *Aphanocapsa* (Apest) and many from *Microcystis* (Mcest). Using the three Apest and randomly selected two Mcest sequences along with the IsPETase, we performed an MSA using the align tab of the UniProt toolbar, which revealed the three catalytic Ser-His-Asp triad typical of alpha-beta hydrolases (Consortium, 2023 and Supplementary Figure S1). PET degrading activity has been reported previously in different enzymes like cutinase, lipase, esterase (Joo et al., 2018; Carr et al., 2020; Maurya et al., 2020) or PETase (an alpha-beta hydrolase fold family member) that act along with MHETase (Palm et al., 2019). These enzymes have been primarily described in bacteria or fungi (Carr et al., 2020; Qi et al., 2021). Although scarce in reports, PET degrading activities are also present in microalgae (Chia et al., 2020). The isolates for this study were green in appearance, and their characteristics under the microscope (Figure 3) resembled much to an extent, the previously reported plastic biodegradation by green photosynthetic microalgae capable of plastic biodegradation (Kumar et al., 2017). Microalgae have been demonstrated with plastic degradation capacity by synthesizing toxins or enzymes while using plastic polymers as carbon sources (Chia et al., 2020).

To assess the ability of isolated green microbes to utilize heat-treated PET films, we subjected them to sodium dodecyl sulfate (SDS) washing to eliminate attached cells before examining them under a light microscope. Despite the SDS wash, both M4 and A2 isolates displayed green growth in connection with PET, indicating growth within the PET films (see Supplementary Table S3). This finding aligns with recent studies that used SEM to demonstrate microalgae adherence to PET surfaces, with alterations noted even after physical and/or chemical pretreatment of PET (Falah et al., 2020, Supplementary Table S3). Interestingly, another study highlighted a preference for nylon over PET, suggesting PET's resistance to biological attachment (Demirkan et al., 2020). Our study involved a comparison of our 400X light microscopy images with previously published SEM scans, revealing green growth within PET films by the isolated green microorganisms (Supplementary Table S3).

PET is a manmade product unlike cellulose or other plant or animal-derived polymers. So, nature needs more time to train its enzyme pool to attack PET; this makes PETases very rare (Carr et al., 2020). Only a handful of reports highlight and mention the PETase enzymes and their activity. A global investigation discovered a significant presence of PET hydrolases in regions with crude oil, underscoring the importance of natural selection (Danso et al., 2018). The current study's enrichment and isolation of PET-utilizing microorganisms suggest that the sampled soils in this research contain life forms adapting to PET consumption.

Nature currently lacks the maturity to efficiently handle manmade PET using microbial enzymes as tools, so a complete understanding of pathways for PET degradation in microbes remains a challenge. Some studies propose the collaboration of microbial consortiums comprising various bacteria, protozoa, and yeast-like cells, which collectively break down PET into TPA and EG. This breakdown allows the cells in the consortium to metabolize these components (Taniguchi et al., 2019). In another study, it was reported that the bacterium *I. sakaensis* can convert PET into CO_2 using two enzymes namely, IsPETase and IsMHETase (Yoshida et al., 2016). Prior to these findings, the fungus *Humicola insolens* cutinase (HiC) demonstrated a preference for producing MHET from BHET. When combined with *Candida antarctica* lipase B (CALB), this system could digest the MHETs into TPA (Carniel et al., 2017). Consequently, there are variations in the breakdown products and the routes that microbes take to degrade PET for utilization. In the future, further biochemical degradation analysis may provide insights into the metabolic routes our isolates are using to utilize PET as their carbon substrate.

3.3 Model of hypothetical protein from *Pycnococcus provasolii* and *Cyanobacterium* TDX16 alpha/beta hydrolase contain active and substrate binding sites for PET hydrolysis

Microalgal PET degradation activity is promising because industrial production of bacterial degrader is costlier than microalgae as the latter do not require organic carbon sources for growth (Moog et al., 2019; Hempel and Maier, 2016). Moreover, endotoxins are absent in algae (Akram et al., 2023). So, PET remediation approaches using algal systems are more appealing than bacterial counterparts. Due to their attractiveness, several works reported the endeavor of heterologous expression of PETase in the microalgal system (Almeida et al., 2019; Moog et al., 2019; Kim et al., 2020) (Table 1). However, naturally occurring microalgal PETases rarely appeared in the literature. Our current report highlights two natural microalgal systems (*Aphanocapsa* and *Microcystis*) with PET-associated growth, which may harbor PETase-like activity. As microalgae offer promise in various aspects, we attempted to explore the presence of naturally occurring PETase genes within microalgae. Our searches were performed by using both BLASTP and TBLASTN (Figure 4). One originates from the organism *Pycnococcus provasolii*, a species of green algae and is a hypothetical protein of 335 amino acids. The solitary, spherical, 1.5-4.0 mm in diameter, resistant, sporopollenin-free, and ultrastructurally close to green algal cells of *Pycnococcus provasolii* are hardly identifiable from the cells of other coccoid planktonic creatures under the light microscope (Guillard et al., 1991).

The other one is cyanobacterium TDX16 alpha/beta hydrolase protein containing 175 amino acids. According to Dong and Xing (2020) the first origin-known alga, TDX16-DE, is synthesized via de novo organelle biogenesis from the *Chroococcidiopsis*-like endosymbiotic cyanobacterium TDX16 after acquiring the DNA of its host green alga, *Haematococcus pluvialis*. With a diameter of 2.0-3.6 m, TDX16-DE is spherical or oval. The strain has 99.7% identity to *Chlorella vulgaris*, according to 18S rRNA sequencing. However, based on several characteristics, including size, membrane-bound organelles, and cyanobacterial origin, scientists established TDX16-DE as a new genus and species, *Chroococcidiorella tianjinensis* (Dong and Xing, 2020).

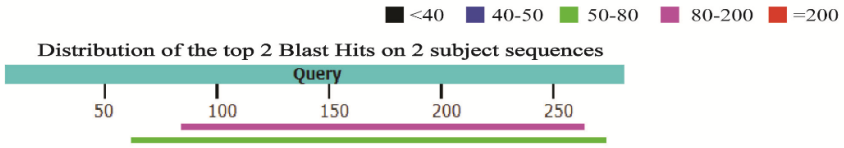
The TBLASTN search also discovered two nucleotide sequences. *Pycnococcus provasolii* genome assembly chromosome 12 is one of them. This gene repertoire, which has 65 protein genes, and 33 RNA genes is comparable in size to that found in *Chlorophyceae* green algae (Turmel et al., 2009). The other one is a partial mRNA of *Emiliania huxleyi* CCMP1516 triacylglycerol lipase 1 (LIP1). Sequencing of CCMP1516 genome revealed its proportion of repetitive components (64%), which is substantially higher than that of sequenced diatoms (Read et al., 2013).

Table 1. Plastic degradation activity in microalgae from literature mining

Organism name	Species name	Detection method	Reference
Green algae	Functional expression of *Ideonella sakaiensis* PETase in *Chlamydomonas Reinhardtii* CC-124	High-performance liquid chromatography (HPLC), Scanning electron microscopy (SEM)	Kim et al. (2020)
Blue-green algae (Cyanobacteria)	Biological degradation of LDPE by *Anabaena spiroides*, and *Navicula pupula*	Weight loss method	Barone et al. (2020)
Microalgae (Single-celled green algae)	PET Degradation by *Chlorella vulgaris* with pre-treatment	Compound microscopy (CM), SEM, Fourier transformed infrared spectroscopy (FTIR), and Gas chromatography-mass spectrometry (GCMS)	Falah et al. (2020)
Mesophilic marine photosynthetic microalga	Expression of PETase in *Phaeodactylum tricornutum*	SDS-PAGE, Western blot, PNGase F assay, SEM, and Ultra high-performance liquid chromatography (UHPLC)	Moog et al. (2019)
Marine sponge-derived strain	Heterologous expression of IsPETase-like gene *Streptomyces* sp. SM14 isolated from the sponge *Haliclona simulans*	*In silico* analysis, polycaprolactone (PCL) plate-clearing assay	Kennedy et al. (2009) and Almeida et al. (2019)

(a)

Description	Total score	Query coverage	E-value	Percentage identity	Accession length	Accessions ID
Alpha/beta hydrolase, partial [*Cyanobacterium* TDX16]	128	62%	3e-34	42.54%	175	OWY58880.1
Hypothetical protein [*Pycnococcus provasolii*]	65.9	73%	6e-10	26.69%	335	GHP05201.1



(b)

Description	Total score	Query coverage	E-value	Percentage identity	Accession length	Accessions ID
Emiliania huxleyi CCMP1516 triacylglycerol lipase 1 (LIP1) Partial mRNA	66.2	67%	2e-10	25.98%	946	XM_005780464.1
Pycnococcus provasolii genome assembly, chromosome: 12	65.1	72%	1e-09	26.75%	879559	OW568872.1

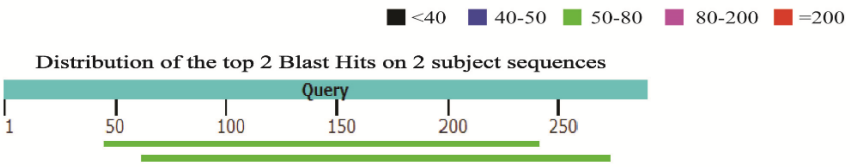


Figure 4. Sequence similarity analysis using BLASTP and TBLASTN for microalgal genes/proteins with PETase-like characteristics using IsPETase protein as the query

In PETase, the catalytic triad comprising Ser160, His237, and Asp206 (Figure 5) indicates a charge-relay mechanism system like that found in other α/β-fold hydrolases. From multiple sequence alignment, a similar active site is found in both microalgal proteins found in the BLASTP/TBLASTN search (Figure 4).

The hydrolytic enzymes of distant phylogenetic origin have been found to contain the alpha/beta hydrolase fold that contains eight beta sheets connected by alpha helices, with a highly conserved catalytic triad than the substrate binding sites. The model of a hypothetical protein from *Pycnococcus provasolii* constructed in this work (Figure 5(c)) also exhibits eight beta sheets that are connected by alpha helices. The Cyanobacterium TDX16 alpha/beta hydrolase model does not show eight beta sheets, which is due to the truncated amino acid sequence (175 AA long) used as a query in the Phyre2.0 server. When fed into the MSA program (Figure 5(b)), both sequences show conservation of the active sites (AS) and substrate binding sites (SBS). Among the nucleophile (serine)-histidine-aspartate loop of the catalytic triad, the histidine does not accommodate any change (Ollis et al., 1992). The substrate binding site has been found to accommodate different amino acids like Phe-Met-(Trp/Tyr/Ala) in Bacteroidetes PET hydrolyzing enzymes (Zhang et al., 2022) whereas Tyr-Met-Trp in *Ideonella sakaensis* (IsPETase) and the leaf compost cutinase (LCC) PETase, etc. In the current MSA analysis, the cyanobacterial protein contains Phe-Met-Trp but the *Pycnococcus provasolii* has Trp-Leu-Asn (Figure 5(b)).

The Cyanobacterium TDX16 alpha/beta hydrolase and *Pycnococcus provasolii* hypothetical protein models shown in Figure 5(c) (i and ii, respectively) were docked with BHET (bis 2-hydroxyethyl terephthalate) dimer. The amino acids predicted to form the SBS and AS are shown in yellow in the ribbon diagram of the models. The binding affinity for BHET dimer against the cyanobacterial protein was -4.9 Kcal/mol whereas for BHET dimer against the pycnococcal protein was -6.5 Kcal/mol. Both of the protein models demonstrate a cleft for binding BHET dimer. A 2D diagram of ligand binding site atoms shows possible interactions including pi-sigma, van der Waals, and H-bonding.

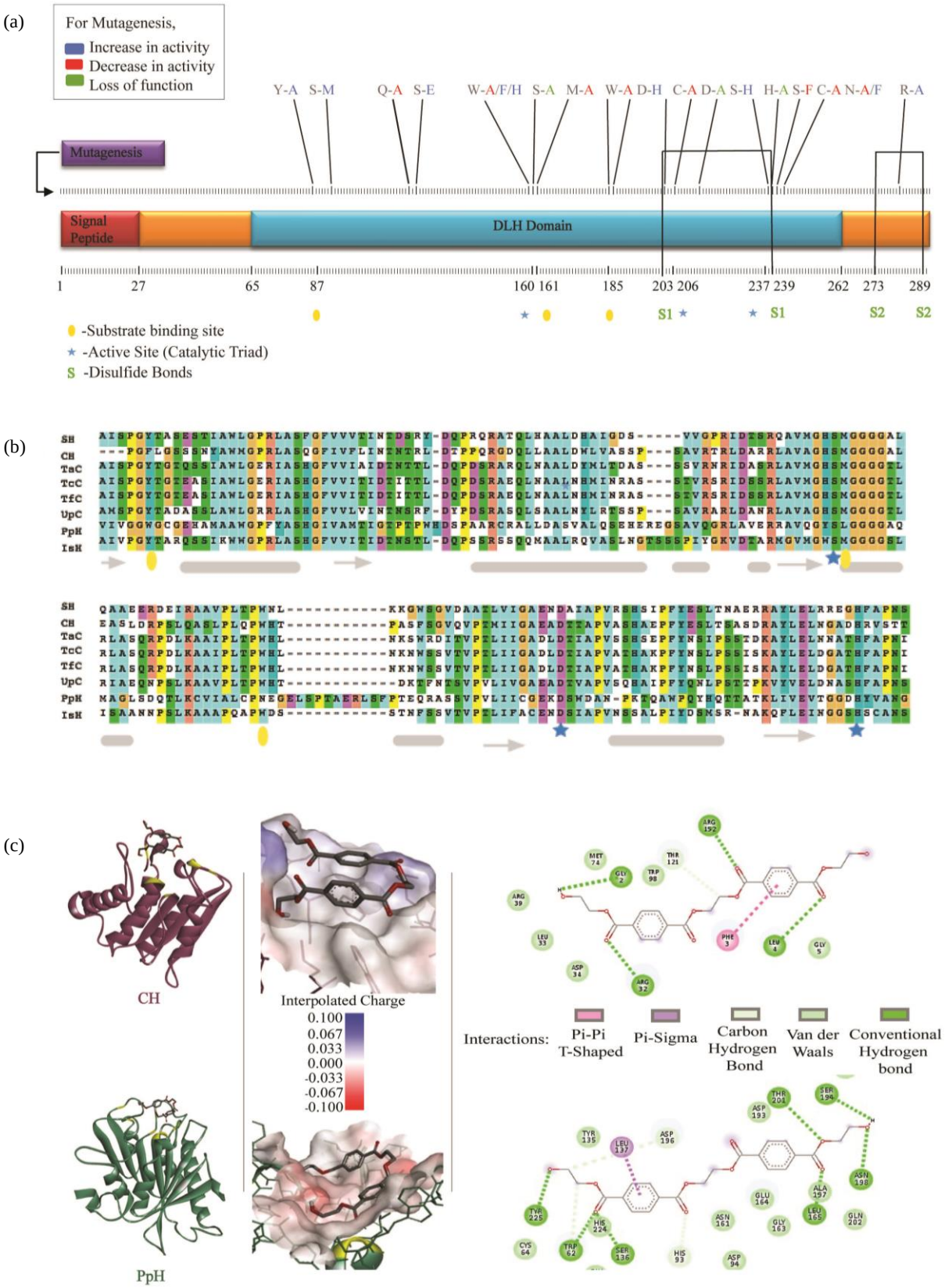


Figure 5. (a) Domain structure of IsPETase showing the highly conserved catalytic triad, substrate binding sites, and (b) disulfide bridges as well as previous mutagenesis effects Homology analysis building MSA. For MSA, eight sequences were used: SH: *Streptomyces* sp. SM14 alpha/beta hydrolase; CH: cyanobacterium TDX16 alpha/beta hydrolase; TaC: *Thermobifida alba* cutinase est1; TcC: *Thermobifida cellulolytica* cutinase 1; TfC: *Thermobifida fusca* Cutinase; UpC: Unknown prokaryotic cutinase; PpH: *Pycnococcus provasalii* hypothetical protein; and IsH: *Ideonella sakaiensis* PET hydrolase. All sequences share the same active site (Ser160, Asp206, and His237) also the SBSs were highly conserved. (c) Model of Cyanobacterium TDX16 alpha/beta hydrolase (i) and *Pycnococcus provasolii* hypothetical protein (ii). The ASs and SBSs are shown in yellow.

4. CONCLUSION

Stopping the immediate use of plastics is highly unlikely, which means we are likely to witness an increasing accumulation of plastic waste over the next decade. Plastics such as PET are commonly used in packaging industries and the production of synthetic fabrics, and their natural resistance to biodegradation has led to a rapid buildup of plastic waste which in turn poses a major threat to various forms of life and, ultimately, to the global climate. This is why it is essential to explore methods for bioremediation of plastics. The issue of plastic waste is a global problem, and it is of particular concern for densely populated delta regions like Bangladesh, which is surrounded by water. Unfortunately, the waste management systems in such areas pay limited attention to and make little effort to address the issue of plastic pollution.

In this study, we explored the garbage disposal site and isolated a few potential PET-utilizing microbes from their natural habitat, the garbage disposal site. In the lab, we screened, cultured and tested their growth utilizing PET as the sole source of carbon at defined conditions, and on synthetic enrichment media. An initial examination and characterization using light microscopy and in silico bioinformatics analysis identified two microalgal isolates with the potential capacity to degrade PET (polyethylene terephthalate). Additionally, when searching protein sequence databases for microalgal PETases, there were indications of possible PETase activity within the microalgal gene pool. However, further genomic and molecular investigations are required for taxonomic classification and to confirm their ability to biodegrade PET. Nevertheless, this study underscores the importance of exploring natural habitats to isolate promising candidates, such as green microalgal cells, with bioremediation capabilities, which could potentially lead the way to a more environmentally sustainable solution for the worldwide plastic crisis in the near future.

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AUTHORS' CONTRIBUTION

Jesmin and MMM conceptually designed the research; SKS and MAM collected, screened and analyzed the data. SKS, SN, MMM, and Jesmin, validate the interpretation and drafted and revised the manuscript. All authors have read and approved the final manuscript.

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