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New approaches for the characterization of plastic-associated microbial communities and the discovery of plastic-degrading microorganisms and enzymes

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ABSTRACT

Plastics in the environment represent new substrates for microbial colonization, and recent methodological advances allow for in-depth characterization of plastic-associated microbial communities (PAMCs). Over the past several decades, discovery of plastic degrading enzymes (PDEs) and plastic degrading microorganisms (PDMs) has been driven by efforts to understand microbially-mediated plastic degradation in the environment and to discover biocatalysts for plastic processing. In this review, we discuss the evolution of methodology in plastic microbiology and highlight major advancements in the field stemming from computational microbiology. Initial research relied largely on culture-based approaches like clear-zone assays to screen for PDMs and microscopy to characterize PAMCs. New computational tools and sequencing technologies are accelerating discoveries in the field through culture-independent and multi-omic approaches, rapidly generating targets for protein engineering and improving the potential for plastic-waste management.

Contents

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1. Introduction

Plastics have experienced unprecedented success in the material industry due to their low cost, versatility, and durability [1]. Mass production of plastics began in the 1950′s and annual production levels now exceed 380 million tons [2]. Plastics often have short service lifespans before disposal. Unfortunately, current recycling practices are limited and only a fraction of plastics are recycled; 79% of plastic waste is discarded in landfills or in the environment [2] where it can cause ecological damage [3–5].

Currently, environmental biodegradation of most conventional plastics, including polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET), has not been observed to any significant degree [6]. Conversely, biodegradable plastics like polyhydroxyalkanoates (PHAs), polylactic acids (PLA) or starch blends compose a small (<1%), but rapidly growing fraction of plastic production and can be metabolized by microorganisms to biomass, CO_2, and H_2O [7,8]. Biodegradation rates depend strongly on environmental conditions including the temperature, humidity, pH, and the microbial community and the enzymes they encode [1]. Compostable plastics are biodegradable plastics that have been certified to decompose into CO_2 and H_2O within a specified timeframe under ideal composting conditions [8,9].

For the past several decades there has been considerable interest in identifying plastic-degrading microorganisms (PDMs) and plastic-degrading enzymes (PDEs). Plastic exposure for most conventional plastics is a relatively recent phenomenon for microorganisms, meaning very limited time has elapsed for the evolution of efficient PDEs for most conventional plastics. While no known enzymes act on high-molecular weight polymers of PP and PS, several PET-degrading enzymes have been identified in the past few decades [6]. Known PET-degrading enzymes are serine-hydrolases with α/β -hydrolase fold with a catalytic triad composed of serine, histidine and aspartic acid and include an esterase from *Thermobifida halotolerans* [10] and cutinases from *Thermobifida fusca*, *T. cellulolysitica* [11], *Humicola insolens* [12] and *Fusarium oxysporum* [13]. In 2016, researchers discovered *Ideonella sakaiensis*, a bacterium cultured from a water-bottle recycling facility that could degrade and assimilate PET via a two-enzyme system [14]. This was the first report of a two-enzyme system apparently evolved for PET-utilization in a PET-enriched environment, indicating microbes may adapt and evolve metabolic pathways to use plastics over time.

In contrast to most conventional plastics, microbes often possess enzymes to degrade biodegradable polymers. For instance, PHAs are naturally made microbial polyesters used as carbon and energy storage compounds that have been commercialized as biodegradable plastics [15]. As a result, certain lineages of microbes possess a suite of enzymes involved in processing PHAs [15]. PHA depolymerases are the key PHA degrading enzymes and are composed of a diverse family of carboxylesterases belonging to the α/β -hydrolase family [15]. PHA depolymerases have a catalytic triad composed of serine (embedded in a GxSxG motif), a histidine, and an aspartic acid with a conserved histidine near the oxyanion hole [16]. PLA however is not a natural substrate for microorganisms and PLA degrading organisms appear to be scarce relative to PHA degrading organisms [17]. PLA degrading enzymes show relatively higher sequence diversity and have been classified as proteases, lipases and esterases [18].

Identifying new PDEs and PDMs initially relied on culture-based approaches involving screening environmental samples for plastic-degrading activity using techniques like clear zone assays, weight-loss measurements, visual observation, and measuring CO_2 evolution [1,17]. While these approaches identified several key PDEs and PDMs, culture-dependent approaches are limited because they overlook a significant fraction of microbial diversity [19]. With the exponential increase in DNA sequencing capacity and maturation of computational biology techniques for annotating sequence data, researchers can now query uncultured microbial lineages from diverse environments. This has enabled high throughput metagenomic screens for PDEs [20]. Total community approaches, or "omics" like metagenomics, metatranscriptomics and metaproteomics, have enabled significant advancements in the understanding of plastic-associated microbial communities (PAMCs) and the microbial ecology of plastic [21]. These methods also identify targets for protein engineering and synthetic biology projects aimed at designing PDEs and PDMs that can efficiently process plastics [22,23].

Many excellent reviews have 1) reported on the enzymes and microorganisms involved in plastic biodegradation [6,15,17,24,25]; 2) described the current knowledge of PAMCs [21,26–29]; and 3) discussed prospects for microbial solutions to plastic waste [30–32]. Shah et al., reviewed classical techniques used to study plastic biodegradation as part of their comprehensive review on biological degradation of plastics [1]. However, with the maturation and popularization of high-throughput sequencing coupled to rapid advancement in computational biology approaches, a review collating and comparing current methods in the field of plastic microbiology was needed.

Here, we review the different branches of plastic-related microbiological research and the evolution of methods from conventional culture-based approaches to high-throughput culture-independent approaches (Fig. 1). Although still in its infancy, we highlight how 'plasti-omics' (multi-omic approach to elucidate plastic-microbe interactions) is revolutionizing the field and leading to a more complete understanding of the impact of plastic waste on the environment. Lastly, we discuss recent protein engineering and synthetic biology research and highlight the critical roles microbes may play in creating sustainable waste-management solutions.

2. Research areas involving microbes and plastics

Understanding the perspectives and goals of current research provides a working framework for discussion of the impact of computational approaches on plastic microbiology as a field. In this section, we broadly categorize research involving plastic and microbes into distinct branches based on the underlying research objectives. We note that these branches are highly connected, and discoveries in one may support or inform findings in another.

2.1. PAMC research

Seeks to characterize the 'plastic microbiome'. In 2013, Zettler et al., coined the term 'plastisphere' to describe life associated with plastic debris [33]. Plastic waste is ubiquitous and provides a new habitat or substrate for microbial growth across environments. This field describes the structure and function of PAMCs and can predict impacts on biogeochemical cycling of nutrients in an environment due to plastic exposure [21]. Prior to the development of high-throughput culture-independent approaches research in this area was very limited, but in the past decade it has become an area of intense and fruitful investigation, particularly in marine environments (see Table 1).

2.2. Plastic degradation research

Is concerned with the mechanisms underlying and the quantification of plastic degradation due to both abiotic and biotic factors [34]. It is important to distinguish between deterioration of

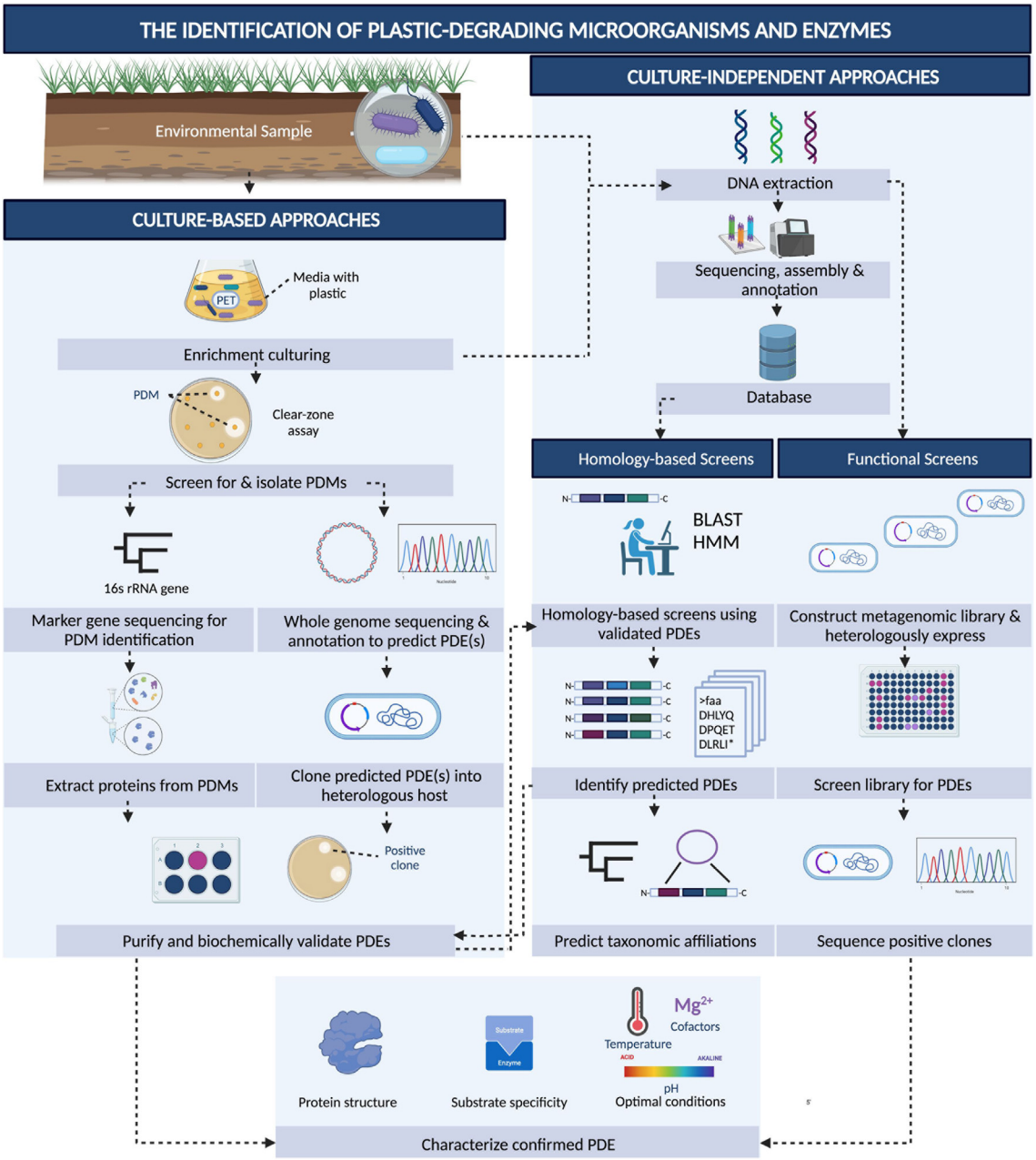

Fig. 1. An overview of approaches for the discovery of plastic-degrading microorganisms and enzymes (PDMs and PDEs). Possible workflows for conventional low-throughput isolate- or culture-based approaches (left) are compared to emerging high-throughput culture-independent approaches including *in silico* homology-based approaches and heterologous host expression-based functional screens (right). Techniques from both approaches can be adopted in order to create a tailored workflow suited to addressing a specific research question or mining a certain environment for PDEs/PDMs. In this context, we use "culture-independent" to refer to techniques or workflows that can capture data from "unculturable" microorganisms. Created with BioRender.com.

the bulk plastic (e.g., fragmentation resulting in microplastics) and depolymerization (degradation of the polymer at the molecular level) [35]. Burial degradation trials involve incubating polymers *in situ* to investigate how plastics behave and/or degrade in different environments over time, which depends largely on polymer properties and environmental conditions [1,36–39]. Standardized tests are used to certify polymers as biodegradable or compostable. According to composting standards (ASTM 6400–19 / EN13432) the plastic must reach 90% conversion to CO_2 under specified conditions within 6 months to be considered compostable [9,40]. Degradation studies can be 'black-box', with no attempt to characterize the microbial community involved, or they can be paired with microbial community profiling or targeted attempts to identify plastic-degraders, depending on the goals of the study. Quantifying plastic degradation is important for the characterization of PDEs and can be done in tandem with microbial community profiling *in situ* or under controlled laboratory conditions. Although relevant and discussed briefly in context of other branches, the full array of methods used to quantify plastic degradation and the factors involved are beyond the scope of this review in which we focus on microbially-mediated plastic depolymerization rather than environmentally-driven deterioration and as such this topic is not covered further.

Table 1
Recent Studies on Plastic Associated Microbial Communities (PAMCs).

Plastic	Environment	Methods	Main Findings	Refs.
PE	Marine	Quantitative staining biofilm assay & clear-zone assay	Microbial biofilms formed rapidly on plastics and found that this correlated with physicochemical changes in plastic properties however, they did not find evidence of PDMs.	[48]
PP, PE	Marine, North Atlantic Sea	Marker gene sequencing & SEM.	Coined the term 'Plastisphere'. Noted that plastisphere communities were distinct from surrounding seawater and harbored a diverse community of autotrophs, heterotrophs, predators, and symbionts. Pits in polymer surface were visualized suggesting breakdown of the polymer.	[33]
PET, PE, PS, and PP	Marine, North Sea	Marker gene sequencing, denaturing gradient gel electrophoresis & SEM	Showed that plastisphere communities varied with season, location and plastic type.	[46]
PE and PHAs.	Marine	Cell counts, 16S rRNA gene sequencing & heterotrophic activity assay.	Showed succession in biofilms differs based on plastic-type: different polymers are initially colonized by similar bacterial communities and then form distinct biofilms with dissimilar diversity and properties.	[49]
PET, PS, PE	Marine	Marker gene, PICRUSt & infrared spectroscopy	Defined a core microbiome on plastics with a distinct metabolism from the microbiome of surrounding water. Found 'keystone species' in the biofilm and 'hitchhikers'.	[53]
Mixed marine plastic debris	Marine	Metagenomics & SEM	Found that plastisphere communities had an enrichment of predicted genes involved in surface-attached lifestyles, nitrogenase genes, and xenobiotic degradation genes and found that microplastics seemed to be autotrophic "hotspots" compared to the surrounding seawater	[68]
PET, PE	Marine, Arabian Gulf.	Marker gene sequencing, Fourier-transform infrared spectroscopy (FTIR) & SEM	Found that fouling was both location and substrate specific. Found more total biomass on wood and steel relative to plastics. Found substrate-specific and location specific bacterial communities. Fissure formation and FTIR spectra indicated plastic degradation by abiotic/biotic factors.	[47]
LDPE	Compost	Marker gene sequencing	Found that the presence of plastics in compost did not have a significant effect on the structure of the microbial community of bulk compounds. Detected changes in interaction patterns of microbial communities between bulk and plastic-associated compost.	[59]
PET and PHA	Marine, benthic	Metagenomics	Found that PET biofilms were non-distinct from a ceramic control, but PHA biofilms were distinct from PET and were dominated by sulfur-reducing microorganisms.	[70]
PE	Landfill	Culturing & marker gene sequencing	Found that different forms of PE plastics select for distinct microbial communities, and that community structure coincides with the plastic's physicochemical properties.	[58]
PBST	Marine	Enrichment culturing, metagenomics, transcriptomics, & proteomics	Obtained a culture that could degrade plastic as the sole carbon source. Identified several novel putative plastic-degrading enzymes. Found that different degradation steps were performed by different community members. Found genomic evidence for functional redundancy for plastic biodegradation, but found only a few were active during biodegradation.	[94]
LDPE, Bio	Soil, wheat rhizosphere	Marker gene sequencing	Identified that biodegradable plastics had significant effect on rhizosphere communities	[54]
PLA/PHA film, 3 types of PBAT-based film, PE	Soil	Enrichment cultures, microscopy, esterase assay & marker gene sequencing	Found PAMCs of mulch films where different when compared to surrounding soil. Found certain lineages were enriched in biodegradable plastic films compared to PE films. Also found a significant increase in esterase activity over time for PHA/PLA and a polybutylene adipate (PBAT)/starch-based film.	[55]
PE	Soil	Metagenomics, culturing & qPCR for ARG detection	Found that soil PAMCs were enriched with antibiotic resistance genes compared to soil communities	[69]

2.3. Identification of new plastic-degraders

Has the specific goal of finding and characterizing new enzymes and /or microbial lineages mediating plastic-degradation and is usually accomplished through screening environmental samples. Motivation for this research typically involves the need for applications for bioremediation and biocatalytic recycling. However, understanding the impact of plastic degradation on nutrient cycling in these environments, particularly in the case of biodegradable plastics, should also be a priority. Screens for new degraders originally relied on culture-based techniques but are now shifting to higher-throughput culture-independent approaches, rapidly accelerating discoveries in the field.

2.4. Design of microbial plastic processing systems

Involves engineering biocatalytic systems for more efficient plastic processing. Protein engineering can modify naturally occurring PDEs to enhance features like biodegradation rates or thermostability to increase their biotechnological applications [20]. Synthetic biology efforts are emerging with engineered plastic-processing microbes capable of improved plastic biodegradation [23], or the 'bio-upcycling' of PET into new polymers including PHAs [41].

3. Characterization of PAMCs or the plastisphere

Initially, taxonomic determination of microbes inhabiting the surface of plastics was limited to observation of different morphologies under a microscope [42]. In 1972, diatoms and bacteria were observed on plastic surfaces from marine environments [43,44]. Now, culture-independent approaches like 16S rRNA gene surveys and metagenomics allow researchers to characterize PAMCs in depth and answer increasingly complex research questions [21].

In 2013, a landmark 16S rRNA gene study showed that PAMCs in marine settings are taxonomically distinct from surrounding water [33]. Further 16S rRNA gene studies have determined that plastisphere microbial communities are shaped by substrate, spatial, and seasonal effects [45–47] and have described successive colonization and biofilm maturation on plastic debris incubated under marine conditions [45,46,48,49]. Computational tools such

as PICRUSt [50], paprica [51], and Tax4Fun [52] were developed to predict functional profiles from 16S rRNA gene amplicon data. Functional predictions from PAMC 16S rRNA gene data showed an enrichment of genes involved in xenobiotic degradation and an underrepresentation of pathways involved in cell motility congruent with an attached lifestyle in marine environments [49,53].

In terrestrial environments, marker gene studies have recently investigated the impacts of plastic agricultural mulch films on microbial communities ([54–56] for a recent review see [29]). Qi et al., found that biodegradable films had a stronger effect on the rhizosphere microbial community than low-density PE (LDPE) and biodegradable films had higher relative abundances of certain bacterial genera including *Bacillus*, *Variovorax* and *Comamonadaceae* [54]. Tanunchai et al., found that adding a biodegradable plastic poly-butylene succinate-co-adipate (PBSA) to soil, microbial communities significantly changed microbial community composition and that archaeal and fungal species richness declined [57].

A landfill 16S rRNA gene study has shown the type and properties of the plastics also impact microbial assemblages: biofilms on plastic waste from a landfill showed that different forms of PE plastics select for different microbial communities, and that community structure is correlated with the plastic's physicochemical properties including dyes and polymer degradation-level [58]. In contrast, a study in compost concluded that the presence of LDPE in compost had no significant effect on the microbial community of bulk compost and that structure of the PAMC did not differ significantly from that of bulk compost [59].

Although significant advances in the understanding of PAMCs have been made with 16S rRNA studies, a single marker gene does not provide a perfect representation of microbial community diversity [60] and functional predictions based on 16S rRNA gene profiles fall significantly short of functional assessments from shotgun metagenomic sequencing [61]. Shotgun metagenomic sequencing catalogues all of the genetic material recovered from an environmental sample allowing for more in-depth characterization and accurate functional profiling of a microbial community. Recovered DNA is sequenced, generating short DNA fragments (reads) which can be computationally assembled into longer scaffolds, and annotated for functional prediction. Advances in long-read sequencing platforms like PacBio are also emerging as promising technologies for metagenomic sequencing producing reads in over 10 kb in length thus simplifying downstream analysis [62]. Metagenome-assembled genomes (MAGs), generated through "binning" algorithms, allow researchers to designate metabolic potential to a specific community member or population [63].

Few studies have applied full metagenomic sequencing to characterize PAMCs to date. Despite plastic waste being prevalent in most environments, most PAMCs research has focused on marine environments [28]. PDMs have been isolated from soil on several occasions [66,67], but comprehensive metagenomic assessments of terrestrial PAMCs have been understudied relative to marine environments. Bryant et al., (2016) showed an enrichment of predicted genes involved in surface-attached lifestyles, nitrogenase genes, and xenobiotic degradation genes in the plastisphere and showed that microplastics seemed to be autotrophic "hotspots" compared to the surrounding seawater [68]. In soil, Zhu et al., (2021) found that plastisphere communities were enriched with antibiotic resistance genes compared to soil [69].

Biodegradable plastic markets are growing in response to environmental concerns with conventional plastics. Biodegradable plastic litter offers a new environmental substrate, one that is more bioavailable than conventional plastics, which may enrich for certain biodegradable plastic-degrading community members. Bandopadhyay et al. observed altered microbial communities in PAMCs of mulch films compared to surrounding soil and enrichments of certain taxa like the *Methylobacterium, Arthrobacter and Sphingomonas* in PAMCs of biodegradable plastic mulch films compared to PE. They also observed a significant increase in esterase activity over time for PHA/PLA and a polybutylene adipate (PBAT)/starch-based film [55]. Pinnell & Turner used metagenomic profiling to show that biodegradable PHA biofilms are dominated by sulfate reducing microorganisms compared to controls [70]. This finding indicates that biodegradable plastic waste deposited in the environment may have unexpected impacts on microbial biogeochemical cycling.

Despite significant advancements in the past decade, the dynamics of PAMCs and the impact of plastics on biogeochemical cycles remain poorly understood. Future studies should employ a 'multi-omic' approach such as metagenomics paired with metatranscriptomics and or metaproteomics with measurements of biogeochemical rates to compare metabolic activities of PAMCs to communities in the surrounding environment (Fig. 2). Multi-omic community profiling should be paired with rigorous attempts to characterize changes in plastic properties in line with degradation trials for a more comprehensive view of biotic factors mediating polymer changes in different environments. Characterization of PAMCs is valuable beyond understanding environmental impacts, as PAMCs are a promising source for novel PDEs with potential biotechnological applications [20].

4. Methods for high-throughput identification or prediction of new PDEs and PDMs

4.1. Metagenomic screens to identify PDEs

Conventionally, PDEs have been identified using culture-based approaches where microorganisms are grown from an environmental sample and screened for plastic-degrading activity (Fig. 1) [17]. Plastic biodegradation can be detected using visual observations [71], weight-loss measurement, changes in polymer properties, CO_2 evolution, chromatography [72] and clear-zone assays [73]. Microorganisms exhibiting plastic-degrading activity are typically enriched in a plastic-containing media and isolated for taxonomic identification [14]. PDEs are conventionally identified using a combination of biochemical and molecular biology approaches [74].

For example, proteins can be extracted from cultured PDMs and tested for plastic-degrading activity. Once isolated, PDE sequence information can be obtained from mass-spectrometry (MS) [74] and biochemical assays can be used to characterize optimal enzyme conditions, and substrate specificity (Fig. 1). Alternatively, once a PDM has been identified, whole genome sequencing and annotation can guide PDE gene prediction [14]. Predicted PDEs can be cloned into a heterologous host to test for activity (Fig. 1).

For example, the PETase from *Ideonella sakainesis* (*Is*PETase) was identified using a conventional culture-based approach by culturing 250 environmental samples from a PET-enriched environment and screening for PET-degrading activity using PET film as the major carbon source. A sediment sample was found to produce morphological changes to the PET-film. Enrichment culturing was used to isolate *I. sakaiensis*. Genome sequencing revealed a putative lipase with shared homology to the hydrolase from *T. fusca* which was purified and confirmed to have PET-degrading activity [14].

Culture-based approaches are advantageous because they can select for and confirm PDMs leading to the identification of several key PDEs. However, culture-based approaches can be labor intensive and are limited since the vast majority of bacterial phyla do not have cultured representatives [19]. This has led bioprospecting efforts for PDEs to focus on the uncultivated microbial diversity [6].

Metagenomic screens are culture-independent methods to identify PDEs, and comprise two general approaches 1)

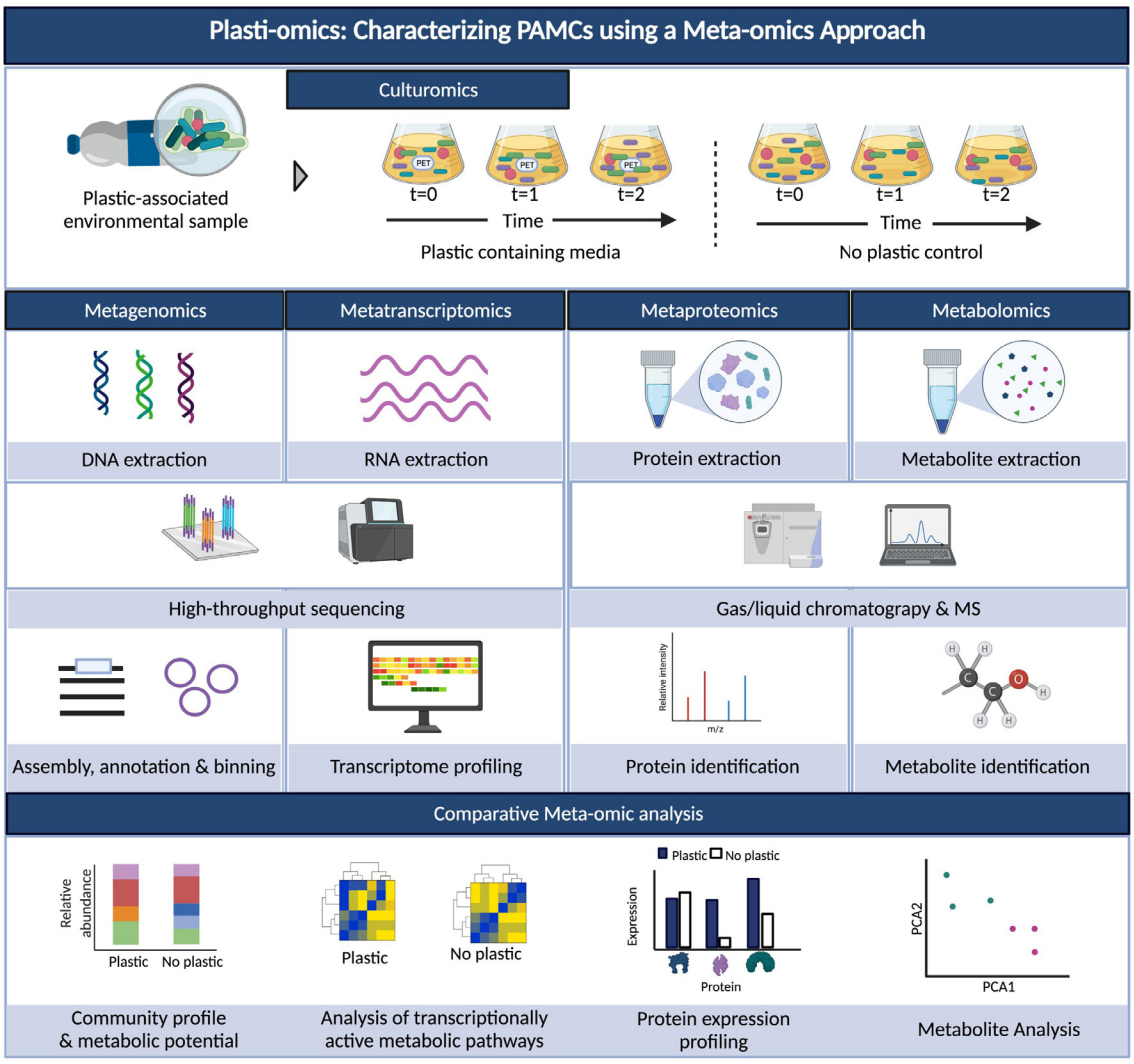

Fig. 2. An overview of *meta*-omic approaches for profiling plastic-associated microbial communities (PAMCs). MS = mass spectrometry. Created with BioRender.com.

homology-based screens and 2) functional screens (Fig. 1). Homology-based screens using hidden markov models (HMMs) or basic local alignment search tools (BLAST) can identify putative PDEs *in silico* based on shared sequence similarity to known PDEs. Homology-based screens of large datasets composed of many metagenomes have identified the global distribution of PET-degrading enzymes [76] and PHA-degrading enzymes [77]. Alongside metagenomic screens, other studies have investigated nucleotide and protein data repositories to predict new PDEs. Using homology-based searches, Knoll et al., created a database of 587 predicted PHA depolymerases identified from NCBI's non-redundant database [16] and Gan et al., created a database of over 8000 predicted PDEs for 22 different polymers [78].

While *in silico* homology-based screens offer a rapid and cost-effective way to screen large amounts of sequence data and greatly expand predicted PDE families, this approach can overlook new enzyme families that do not share sufficient sequence similarity to known enzymes. Biochemical validation is required to confirm activity of predicted enzymes since sequence-similarity does not guarantee shared function, as mutation can alter or impede enzyme function. Furthermore, PDE expression should be confirmed using RNA/protein analysis under condition of interest. The high-throughput nature of homology-based screens means they extraordinarily outpace labor-intensive functional confirmation and protein characterization, leaving an imbalance between predicted PDEs and PDEs with confirmed functions. Guided by bioinformatic analysis, directed efforts that identify new putative PDE clades from this abundance of predicted PDEs offer the opportunity to identify enzymes with novel features or from microbial lineages not previously implicated in plastic biodegradation. The development of effective computational algorithms to identify PDEs and parallel development of consistent, higher-throughput functional assays to confirm computational predictions will be important for future investigations [6]. Early work in this direction has shown promise: Hajighasemi et al. (2018) used medium-throughput screens of 200 hydrolases from environmental metagenomes to identify ten proteins with strong hydrolytic activity against synthetic polyesters [79].

Functional metagenomic screens involve cloning DNA extracted from an environmental sample into a fosmid or cosmid clone library and then transforming that library into a heterologous host for expression and functional screening (Fig. 1) [80]. Unlike homology-based screens, functional metagenomic screens confirm desired plastic-degrading phenotypes and do not rely on *a priori* sequence information, allowing discovery of previously unknown enzyme families [20]. Functional metagenomics provides a powerful high-throughput approach to study gene function. However, library construction can be expensive and technically challenging,

often resulting in substantial loss of DNA or challenges associated with heterologous expression [80]. Nonetheless, functional metagenomic screens have emerged as a successful approach to identify novel PDEs. Using functional metagenomics, Mayumi et al., (2008) identified three novel poly-lactic acid (PLA) depolymerases from compost [18]; Popovic et al. (2017) identified seven polyesterases that could degrade either PLA or polycaprolactone (PCL) [81]; Tchigvintsev et al. (2015) identified five cold-adapted polyesterases [82] and Sulaiman et al. (2012) identified a cutinase from leaf branch compost that could degrade PET [83].

One disadvantage of a metagenomic approach is that it is not always possible to identify the taxonomic origins of a PDE. Genome resolved metagenomics can implicate new PDE-encoding lineages. Despite major advancements, large fractions of metagenomes are often unbinned [84] or occur in poor quality MAGs (<50% complete) [85]. In these cases, the taxonomic origin of a PDE-encoding scaffold can be predicted using computational tools such as MEGAN [86], which has been used to predict new PET depolymerase- [76] and PHA depolymerase-encoding lineages [77] from unbinned metagenomic data.

Most metagenomes screened to date originate from natural environments, revealing generally low numbers of predicted PDEs [6]. This may be a product of overrepresentation of marine and terrestrial (soil) metagenomes in databases like the Integrated Microbial Genomes (IMG) [87]. Plastic-enriched sites such as landfills and PAMCs are understudied and may offer a more promising source of novel PDEs due to adaptive evolution [20,58]. Compost has also proven to be a valuable source of PDEs, likely due to the abundance of recalcitrant biopolymeric substances [18,83]. Accelerating the biodegradation of PLA and PHAs in compost is of particular interest, given these compounds are expected to be diverted from general waste streams to composting facilities. Research on compost PAMCs may help elucidate key PDMs and lead to development of optimized plastic-degrading microbial communities to accommodate increases in biodegradable plastic waste.

Functional or sequence-based screens may not capture low abundance community members who harbour PDEs. These PDMs may be enriched with exposure to a plastic substrate, facilitating their identification (Fig. 1). Pinnell and Turner (2019) observed an increase in PHA depolymerase genes in a benthic microbial community in response to PHA substrate availability compared to controls [70]. Researchers may be able to enrich for PDE-encoding microbes by amendment with plastic substrates, and then pair metagenomic community profiling with techniques such as stable-isotope probing (SIP) using heavy-isotope-labelled plastics to track plastic biodegradation. This workflow could increase the likelihood of identifying novel enzymes and processes by elucidating the microbes encoding PDMs [88]. While approaches such as weight loss measurements and changes in polymer properties can indirectly indicate polymer biodegradation, SIP has been used to unequivocally show the incorporation of polymer-labelled carbon into microbial cells inhabiting the polymer surface of biodegradable or nano plastics [89,90]. Although advantageous for linking metabolic activity with taxonomy, limitations of SIP include cost, complications due to metabolic cross-feeding and issues with sensitivity or incubation times [91,92] which may limit applications particularly for non-biodegradable plastics which are not incorporated into biomass to any significant degree.

4.2. Other 'omics: Insight from RNA and proteins

Metatranscriptomics and metaproteomics are large-scale examinations of the transcribed and/or expressed fraction of microbial genes. These approaches can reveal the metabolic pathways active under different conditions and can confirm functional predictions from metagenomic screens demonstrating expression of predicted or confirmed PDEs. Proteomics has shown huge potential for mining new enzymes for biotechnological applications. For example, studies have applied multi-omic approaches to reveal both community level and single organism responses to polycyclic aromatic hydrocarbons (PAHs) to help elucidate the mechanisms of biodegradation [93]. Likewise, targeted and carefully controlled experiments comparing microbial communities in the presence and absence of plastics can reveal metabolic pathways or proteins involved in mediating plastic-microbe interactions, including proteins involved in biodegradation [94]. Screening the exoproteome is particularly promising for the identification of PDEs since these proteins are secreted from cells and can interact with high-molecular weight polyme [20]. At this time, although comparative proteomics has been applied to isolated organisms, studies applying metatranscriptomics or metaproteomics to plastic degradation trials are rare. Meyer-Cifuentes and colleagues (2020) applied a multi-omics approach (metagenomics, metatranscriptomics and metaproteomics) to a marine consortium incubated with plastic as the sole carbon source. The analysis identified numerous putative PET-degrading enzymes and allowed the authors to propose a mechanism for polybutylene adipate terephthalate-film biodegradation [94]. The power of multi-omic approaches and advanced computational analyses has not yet been realized in microbe-plastic research.

5. Design of microbial plastic processing systems: Protein engineering and synthetic biology to enhance plastic biodegradation

High substrate-specificity and mild reaction condition requirements make enzymes an enticing prospect for applications in sustainable waste management and the biocatalytic recycling of plastic waste [30,95]. Most PDEs are not naturally suitable for biotechnological applications due to low turnover rate and low thermostability [6,20], but protein engineering can be used to enhance or tailor enzymes to improve their function in industrial contexts. Rapid algorithm development has improved the ability to manipulate structures and functions of enzymes [96], enabling faster, more accurate identification of modification targets.

Engineering PET-degrading enzymes with new features or enhanced degradability has been a topic of intense investigation. Since the 2016 discovery of the *Is*PETase, several groups have characterized the structure and key catalytic residues [97,98] leading to subsequent protein engineering efforts. Protein engineering through rational design uses prior knowledge of protein structure coupled with computer simulation and modeling to modify the protein of interest [20,99]. Engineering experiments on the *Is*PETase have enhanced activity, with improved PET and polyfuranoate (PEF) degradation [100]. Cui et al. (2021) devised a computational strategy to redesign the *Is*PETase to a 31 °C higher melting temperature and over 300-fold increase in the degradation of semi-crystalline PET [96]. Others have designed a chimera of the *I. sakainesis* two-enzyme system including the *Is*PETase and downstream MHETase enzyme, which cleaves mono(2-hydroxyethyl) terephthalate into terephthalic acid and ethylene glycol, to improve PET biodegradation [101]. Tournier et al. (2020) engineered the PETase identified from leaf-branch compost to improve thermostability and PET-depolymerization, and subsequently used the resulting monomers to resynthesize PET. This 'proof-of-concept' design demonstrates the possibility of biocatalytic recycling, or a circular plastic economy [22].

Synthetic biology aims to engineer or redesign existing biological systems to accomplish targeted goals. Computational biology streamlines this process, allowing incorporation of environmental sequence data to the theoretical design stage [102]. Moog et al.

(2019) genetically-modified a microalgae to secrete PDEs and mediate PET degradation [23]. Tiso et al. (2021) demonstrated the biocatalytic upcycling of PET to PHAs through sequential enzymatic degradation of PET followed by PHA production using the liberated monomers in a modified *Pseudomonas* species [41]. Further work has focused on refining the assays for assessing PETase activity, including engineering phage display proteins to localize *Is*PETase on the expression host's cell surface [103]. Although still early, synthetic biology efforts are accelerated by computational approaches and rapid advancements in the understanding of PAMCs and discovery of PDEs. Synthetic biology represents an exciting area of innovation that may one day provide sustainable waste-management tools.

6. Summary and outlook

Plastics represent a global environmental concern. For decades there has been substantial interest in the biodegradation of plastic and microbial communities inhabiting plastic debris. Biodegradable plastics offer a growing alternative to conventional plastics and understanding the mechanisms of how microbial communities respond and mediate the degradation of plastics in different environments is critical as these plastics grow in popularity. However, up until recently, techniques to probe these plastic-microbe interactions remained limited. Plasti-omics, the application of high-throughput computational biology approaches to characterize PAMCs and identify PDEs and PDMs has led to exciting advancements even while in its infancy. Technological advances paired with the development of computational tools allow efficient analysis and management of large datasets. Although researchers have started to employ metagenomic screens as a routine search for novel PDEs and PDMs, powerful techniques such as functional metagenomics, SIP, metatranscriptomics and metaproteomics have not yet been widely adopted to elucidate plastic-microbe interactions. These approaches can be technically challenging and expensive, calling for more collaboration between researchers with expertise in the fields of plastics degradation, environmental microbiology, and computational biology. In conclusion, interdisciplinary approaches leveraging advances in computational biology paired with conventional culture-based techniques have the potential to design sustainable, effective plastic recycling pipelines, addressing the global issue of plastics overuse and pollution.

CRediT authorship contribution statement

V.R. Viljakainen: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **L.A. Hug:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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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