Plastic Recycling Using Waxworms: Biotechnology Solutions

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Abstract

As small particles that do not degrade, microplastics harm the environment. Plastics are physically broken down rather than chemically during the traditional recycling process. An insect naturally found in honeybee hives known as the waxworm can break down the most common plastic: polyethylene. A literature review was conducted on a new method to recycle plastics involving the waxworm *Galleria mellonella*. Literary review studies suggest that recycling using waxworms is more efficient and faster than traditional methods due to oxidation by enzymes. After conducting a literature review, a bioinformatics study was carried out using published DNA and protein sequences. Two promising enzymes named Ceres and Demetra were identified from literature and compared to others using multiple sequence alignment. This data was then combined with the 3D model of the enzyme to infer properties and a potential mechanism of biodegradation. By identifying enzymes common to insect and bacterial species, microorganisms may be selected for use in plastic recycling and grown on commercial scales. Soil remediation using this method is possible, reducing harmful microplastics from agriculture and streamlining the recycling process. These findings reveal that in the aerospace domain, production of fuel is possible from products of plastic recycling. Food may also be sustainably grown in artificial habitats on a crewed Lunar or Mars mission by reusing materials in situ.

Keywords: recycling, microplastic, waxworm, biotechnology, In Situ Resource Utilization

Figure 1. Greater wax moth, *Galleria mellonella*, dorsal view. Credit: Simon Hinkley & Ken Walker, Museum Victoria (licensed under the Creative Commons Attribution 3.0 Australia license).

Background

- Microplastic – plastic particles smaller than 0.5 mm
  - Primary: exfoliants in soap (banned in U.S.)
  - Secondary: breakdown of material (Nguyen, 2023)
- 94% of U.S. tap water contains plastic fibers
  - 54% is polypropylene (Nguyen, 2023)
- Newer/safer chemical upcycling methods, i.e. neutral pH & room temperature
  - Plastic production expected to double by 2050 (Zhang, 2022)
- Biodegradation of polyethylene, PE
  - *Pseudomonas* bacteria isolated from solid waste disposal (Pathak, 2023)
- Enzymes in waxworm larva for PE breakdown (Saluis-Verdes, 2022)
  - +Bacteria in gut (Montazer, 2021a)

#1	#2	#3	#4	#5	#6	#7
PET/ PETE	HDPE	PVC	LDPE	PP	PS	Other
Polyethylene tetra-phthalate	High-Density Polyethylene	Polyvinyl Chloride	Low-Density Poly-ethylene	Poly-propylene	Poly-styrene	Bisphenol A (BPA), Polycarbonate, bioplastics
Disposable food containers	Household cleaner bottles	Children's toys, Detergent/ shampoo bottles	Shopping bags	Straws	Styrofoam cups	Miscellaneous

Figure 2. Table of different types of recyclable plastics. Lower numbers are easier to recycle, while higher numbers are more difficult (U.S. DOE, Office of Energy Efficiency and Renewable Energy, 2021).

Methods

- Literature review – waxworm salivary enzymes
  - Ceres XP_026756459.1, Demetra XP_026756396.1 (Saluis-Verdes, 2022)
- NCBI BLASTP, excluding results from *Galleria mellonella*
  - Multiple sequence alignment, ClustalX version 2.1
  - Visualized in Jalview version 2.11.2.7
- 3D structures of waxworm proteins seen with Expasy Uniprot
  - Alphafold can highlight single amino acids
  - Protparam tool shows protein parameters
- Literature review – waxworm gut microbes (Montazer et. Al, 2021a)
  - *Lysinibacillus fusiformis* MH321607.1
  - *Priestia aryabhattai* MH321608.1
  - *Microbacterium oxydans* MH321609.1
- Bacterial 16S RNA comparison: PE degraders (Hou, 2022; Pathak, 2023)
  - *Pseudomonas aeruginosa* MN889032.1, MN889034.1, KT860423.1
    - *P. knackmussii* MN889421.1
    - *P. nitroreducens* MN889035.1
    - *P. putida* KT860422.1
- Phylogenetic tree made in Jalview using DNA sequences
  - Neighbour Joining, but not Principal Component Analysis selected

Literary Review Data

- Polyethylene (PE) Biodegradation is multifactorial in waxworm *Galleria mellonella*
  - Salivary enzymes, Ceres and Demetra
    - Confirmed microbe-free through electron microscopy (Sanluis-Verdes et. Al, 2022)
    - Can oxidize PE without any pre-treatment (Sanluis-Verdes et. Al, 2022)
      - Gas Chromatography-Mass Spectrometry / GC-MS
      - High-Temperature Gas Pressure Chromatography / HT-GPC
      - RAMAN Shift
  - Gut bacteria, separate mechanism
    - Weight loss of PE (Montazer et. Al, 2021a)
      - Mixture of all 3 bacterial cultures outperformed any in isolation
    - Total biomass production (Montazer et. Al, 2021a)
      - 0.25 ± g/L cdm individually; 0.33 ± 0.026 g/L in mixed culture
- Environmental microorganisms and PE biodegradation
  - Bacteria
    - Solid waste disposal site: *Bacillus subtilis*, *P. aeruginosa* (Pathak, 2023)
    - Wastewater facility: *P. aeruginosa* (Hou et. Al, 2022)
  - Fungi
    - Variable: up to 51% in *Aspergillus oryzae* with prooxide (Nguyen et. Al, 2023)
    - Mixed fungal culture: higher PE weight loss than isolated fungus (Nguyen et. Al, 2023)

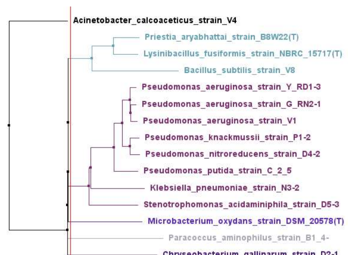

Figure 3. Phylogenetic tree of bacterial strains known to be capable of PE degradation, made in ClustalX and seen in Jalview. Multiple sequence alignment was made using bacterial 16S DNA.

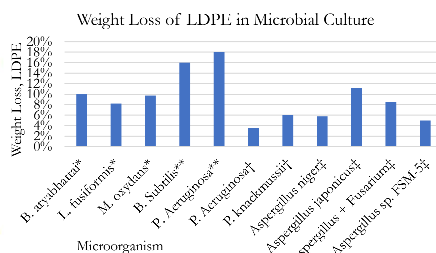

Figure 4. A column chart showing the biodegradation of low-density polyethylene, LDPE, by various microbes. Single asterisk (*) reported by Montazer et. Al after 18-day incubation; double asterisk (**) by Pathak after 4 months incubation; dagger (†) by Hou et. Al after 8 weeks incubation; double dagger (‡) by Nguyen et. Al, variable period.

Bioinformatics Analysis Discussion

- Ceres & Demetra, saliva proteins from greater wax moth *Galleria mellonella*
  - 1st animal enzyme capable of PE biodegradation (Sanluis-Verde et. Al, 2022)
  - No pretreatment necessary for PE oxidation (Sanluis-Verde et. Al, 2022)
    - Bottleneck step for recycling (Zhang et. Al, 2022)
  - Similarity to protein in lesser wax moth *Achroia grisella*
    - Uncharacterized protein, Indian meal moth *Plodia interpunctella*
      - Capable of PE biodegradation: gut bacteria (Pathak, 2023)
- Multiple Sequence Alignment
  - Highly conserved region from amino acids 500-580
    - Antiparallel beta-sheets in cylinder: β-barrel
    - Pfam entry PF03723 – Hemocyanin, ig-like
    - Serine 518 could be part of serine protease
- Protein Folding
  - Low confidence regions
    - Chaperone protein needed for folding
    - Other protein subunits binding site
    - Multiple independent conformations
- Amino acid sequence for Ceres/Demetra only a predicted set
  - Based on PCR and sequencing (Sanluis-Verde et. Al, 2022)

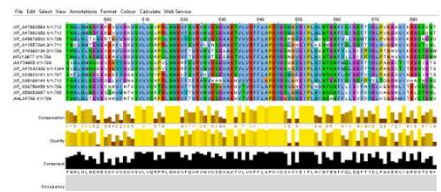

Figure 5. A multiple sequence alignment of a portion of Ceres, an enzyme found in waxworm saliva. Accession numbers from NCBI are shown in the far left; conserved amino acids are highlighted in various colors.

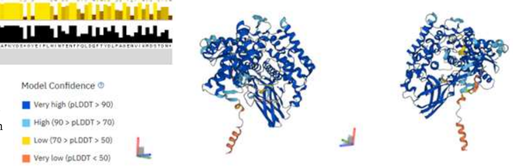

Figure 6. A simulated view showing the 3-D structure of Ceres using Alphafold. Regions of the protein shown in dark and light blue are relatively high confidence, while yellow and orange are lower confidence.

Potential Impacts

- Microplastics in the ocean
  - 92% of ocean debris micro- and nano-plastics, 2018 (Nguyen et. Al, 2023)
  - Seabed: 14 M tons, 2020 (Nguyen et. Al, 2023)
  - Plastic pollution in 5 countries outweighs all others (Nguyen et. Al, 2023)
    - China, Thailand, Indonesia, Philippines, Vietnam
- 75% plastic waste mismanaged, East & Southeast Asia (Nguyen et. Al, 2023)
- Biofilm formation
  - Mixture of bacterial species outperformed any in isolation
    - *C. necator, P. putida* (Montazer et. Al, 2021)
  - Mixed fungi had higher PE weight loss than isolated culture
    - *Fusarium, Aspergillus* (Nguyen et. Al, 2023)
  - No bacterial PEase gene found in study
    - Further research needed – PCR can be run *in silico*
- Agriculture – food on Mars
  - PE is used as mulching to retain moisture (Hou et. Al, 2022)
  - Breaks down physically, producing microplastics (Hou et. Al, 2022)
- Aerospace – fuel production
  - Recycling plastics produces solid waxes, liquids, gases (Zhang, 2022)
    - Avoid Carbon Dioxide release (Zhang, 2022)

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