SCIENTIFIC ENGLISH IMMERSION WITH HANDS-ON EXPERIMENTATION ON MICROBIAL PLASTIC DEGRADATION IN VIETNAM

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Citizen Diplomacy Action Fund (CDAF)
2024

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Long An, Vietnam

TTU
Virtual
July 20, 2024

ASSIGNMENTS

Reminders

- Write a scientific manuscript in English based on published literature and on actual experimentation. **Due Friday, Aug 9, 5 pm**. Link will be provided. Late submission will not be considered.
- Write a proposal ‘to secure a grant’ about protecting the environment (300 words max). Project proposal should contain an overview statement, project goals, target audience, and expected outcomes. **Due Friday, Aug 2, 5 pm**. Link will be provided. Late submission will not be considered.

AI

- What is AI?
- How does it work?
- Did you use it already?

ARTIFICIAL INTELLIGENCE (AI)

- Intelligence exhibited by machines or computers
- Softwares that can perceive the environment and utilize intelligence to take actions maximizing chances of achieving the goals
- Historically,

  → Alan Turing (English mathematician) - first to conduct research in machine intelligence; worked for British government to decipher German codes during WWII

  → 1956 – AI started as an academic discipline by the founding fathers of AI or Information Age (John McCarthy, Marvin Minksy, Claude Shannon, and Nathaniel Rochester)

  → 2012 – deep learning research vastly intensified

  → 2020s – AI boom; US universities, companies and laboratories pioneered significant advances in AI

IMPACT OF AI

Societal and economic shift towards increased automation, data-driven decision making and AI integration into economic sectors:

- Job markets
- Healthcare
- Education
- Government
- Propaganda
- Disinformation

Long-term goal:

→ For AI to complete any task performed by a human being on an at least equal level.

GPT

Generative Pre-trained Transformers

- Large language models
- Based on semantic relationship between words in sentences
- Pre-training consists of predicting the next token (word)
- Accumulate knowledge about the world and generate human-like text
- But, generate falsehoods ('hallucinations'): reduced by reinforced learning by human feedback (RLHF)
- Use chatbots (chatterbot): web interface to mimic human conversation through simple texts or voice interactions
- Process data (modalities): text, images, videos, sound

Current Models and Services:

- **ChatGPT, Grok, Claude, Copilot**
- **Mistral AI** (from Meta Platforms and Google DeepMind)

AI IN THIS PROJECT

?

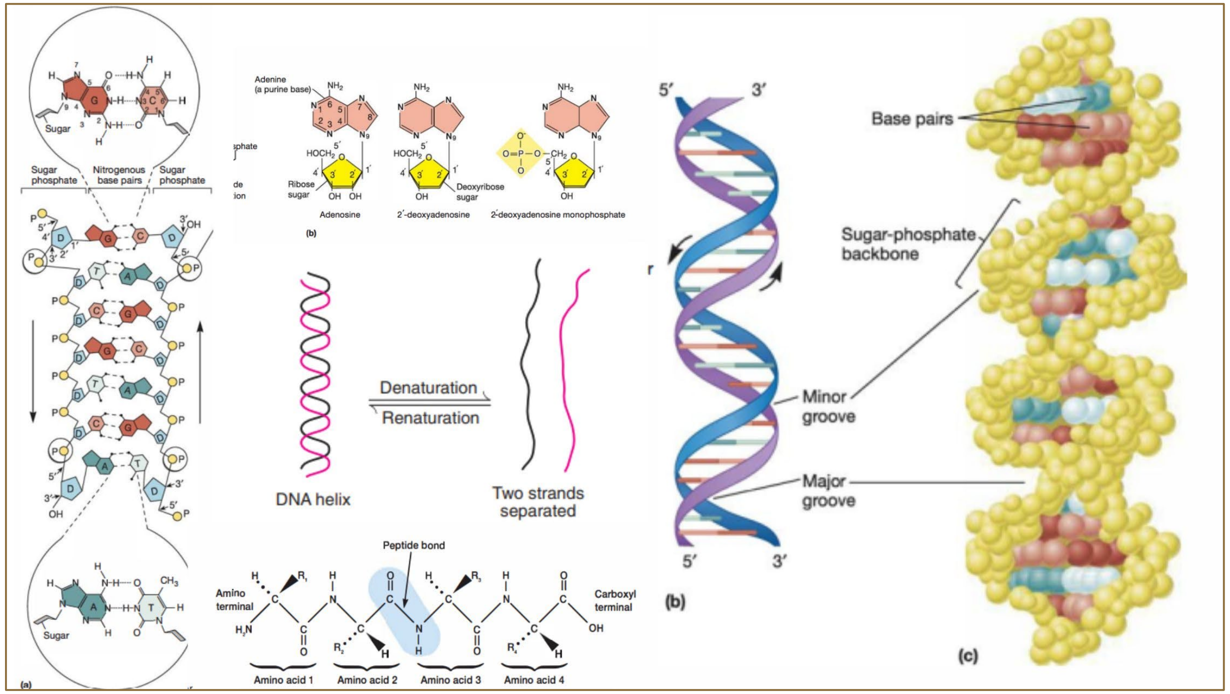
KINDS OF POLYMERS

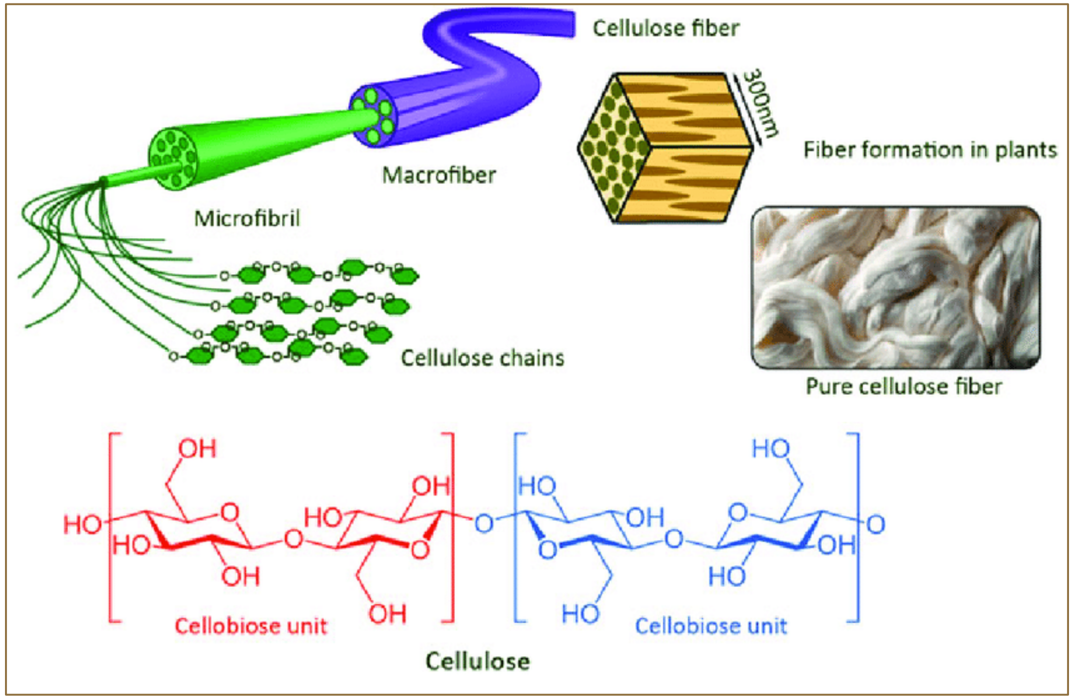
- Synthetic: man-made
  Example: plastic

- Natural: made by nature
  Example: nucleic acids, carbohydrate, protein

NATURAL POLYMERS

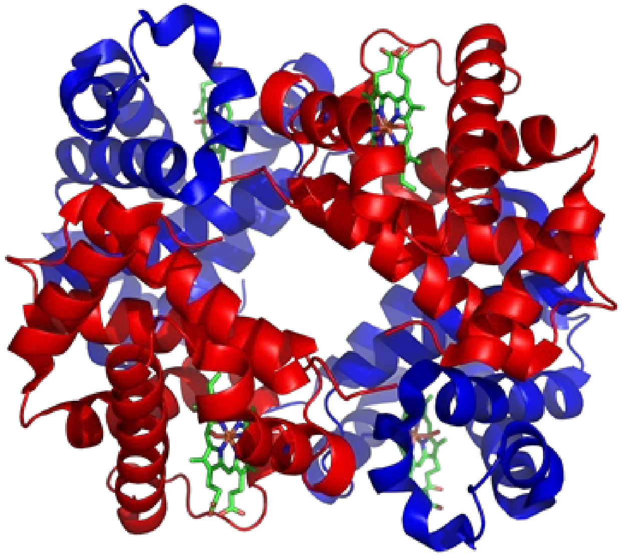
- Nucleic acids: DNA and RNA
- Carbohydrates: starch (rice, potato), cellulose, glycogen





FUNCTIONS OF PROTEINS

- Hormonal – Example: insulin (regulate blood glucose level)
- Transport – Example: hemoglobin (transport oxygen in the body)
- Storage – Example: ferritin (store iron)
- Structural – Example: collagen (in the skin), keratin (in the hair)
- Catalytic – Enzymes (example: trypsin - hydrolysis or cleavage of protein into small components)



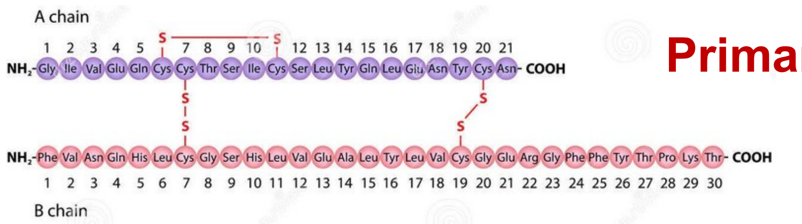
STRUCTURE OF INSULIN

Organizational Level of a Protein

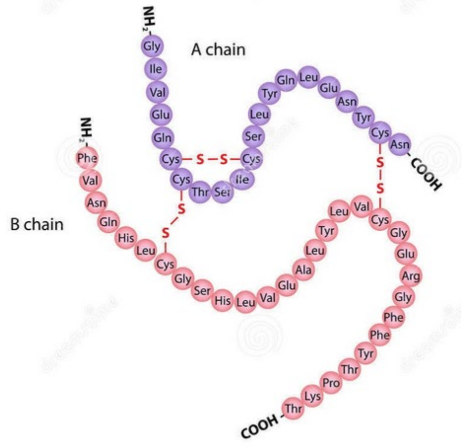
- Primary
- Secondary
- Tertiary
- Quaternary

Insulin (pancreatic hormone)

- first protein to have its primary structure determined by Sanger (1958 Nobel Prize)

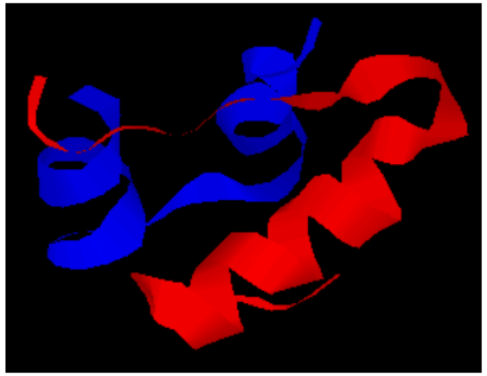


Primary structure



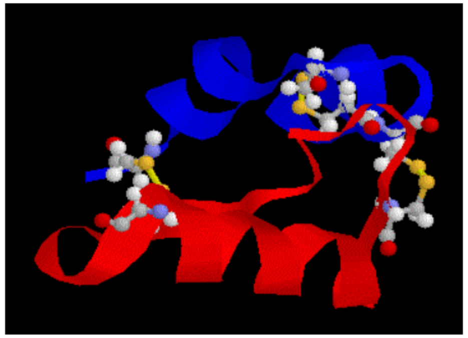
Insulin

Secondary



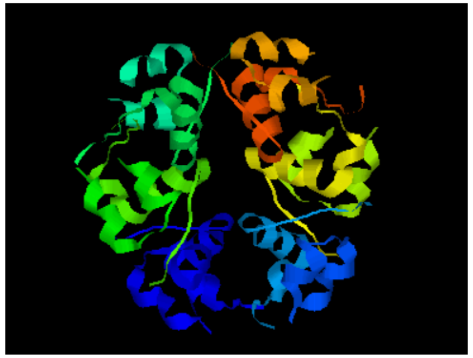
The A chain is shown in blue, the B chain is shown in red.

Tertiary



Note the 3 disulphide bridges (yellow) above, between pairs of cysteine residues (shown as ball and socket diagrams projecting from the ribbons): One within the A chain can be seen at the top, and two between the A and B chains, are at either side.

Quaternary



ORGANIZATION OF PROTEINS

Organizational Level of a Protein

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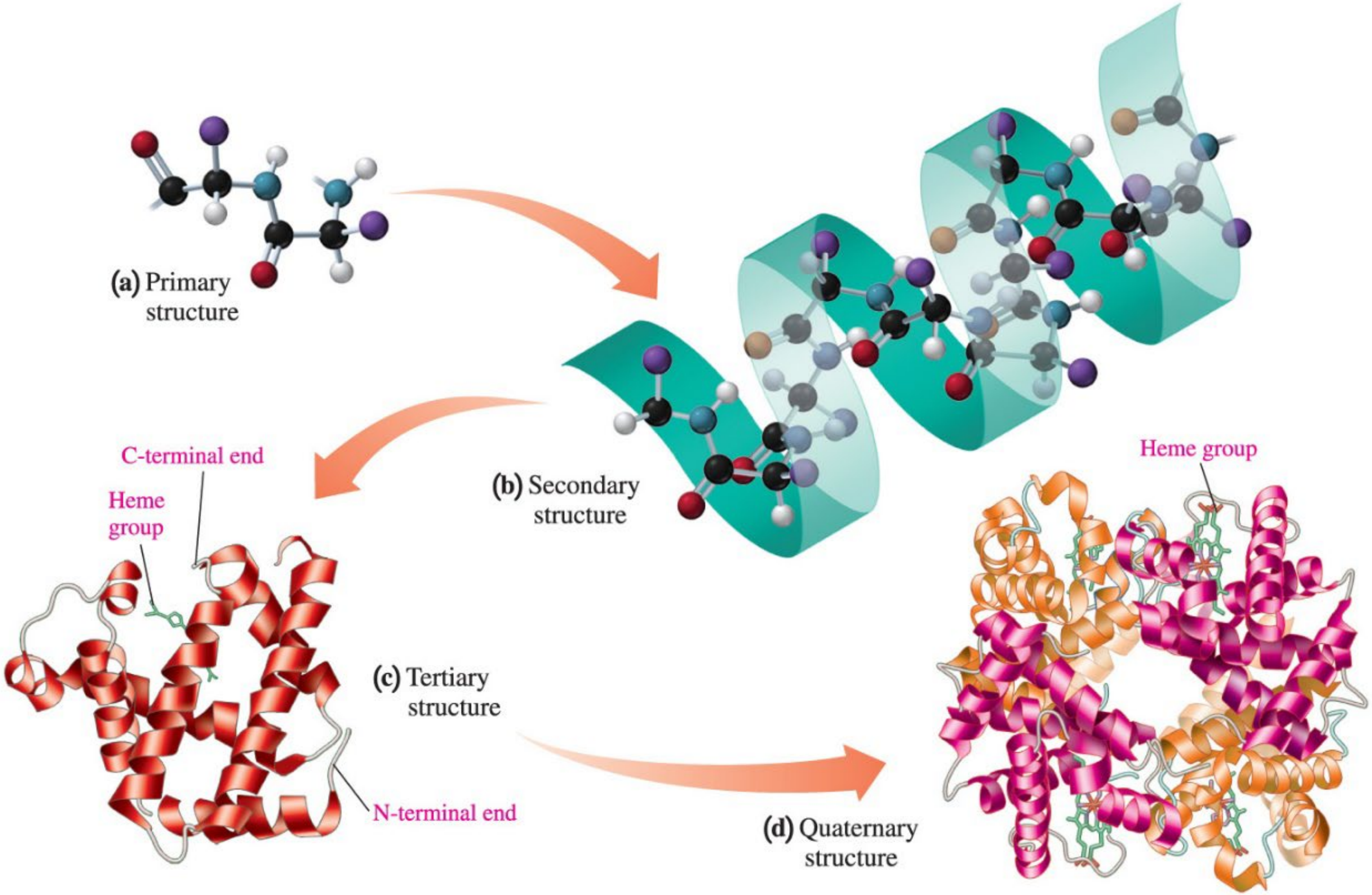
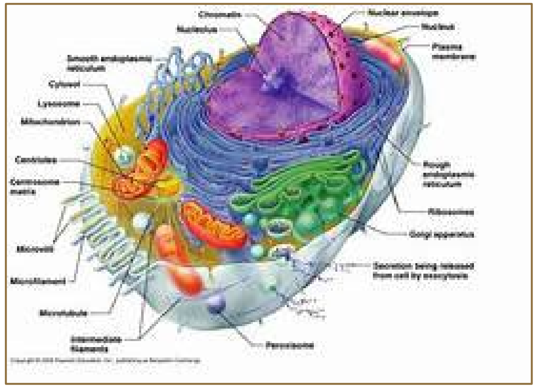


UNDERSTANDING PROTEINS

- Understanding proteins can unlock the mystery in our living activities. Proteins are key players in our life. They work together in a complicated and coordinated way to support life on this planet.
- Knowing the functions of proteins can provide some answer to the fundamental question of humankind and that is, ‘What is life?”
- Observing the structure of proteins in detail could give insights on how they work.
- Structural Biology → deals with structural analysis of living matter at every organizational level; study of protein function based on protein structure; helps understand **LIFE AT THE MOLECULAR LEVEL**.



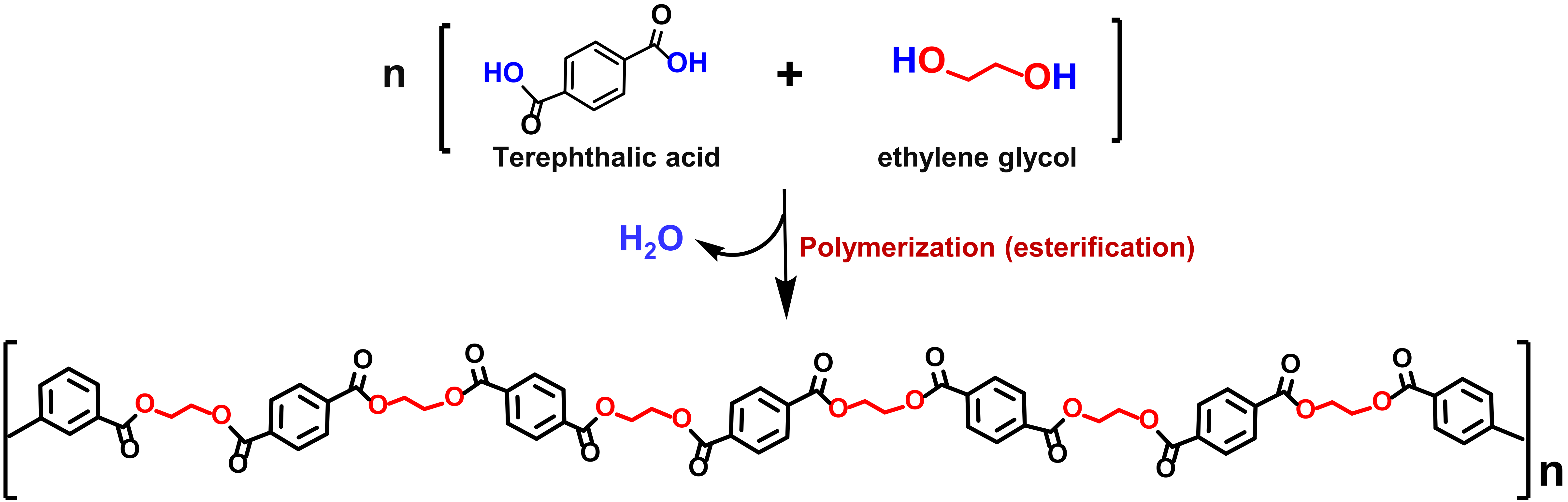
POLYETHYLENE TEREPHTHALATE (PET)

Polyethylene terephthalate (PET):

- Common thermoplastic polymer resin of the polyester family
- Used in fibers for clothing, containers for liquids and foods
- Electronics, X-ray sheets, photographic applications
- Thermoforming for manufacturing
- Engineering resins in combination with glass fibers

- **Historically,**
  → **Polyester:** first invented and patented by JR Whinfield (English chemist) in 1941
  → Commercialized under the name Terylene by ICI (1940s)
  → DuPont launched the name 'Dacron' in the 1950s
  → Used in textile industry (thermal wear, sportswear); automotive industry

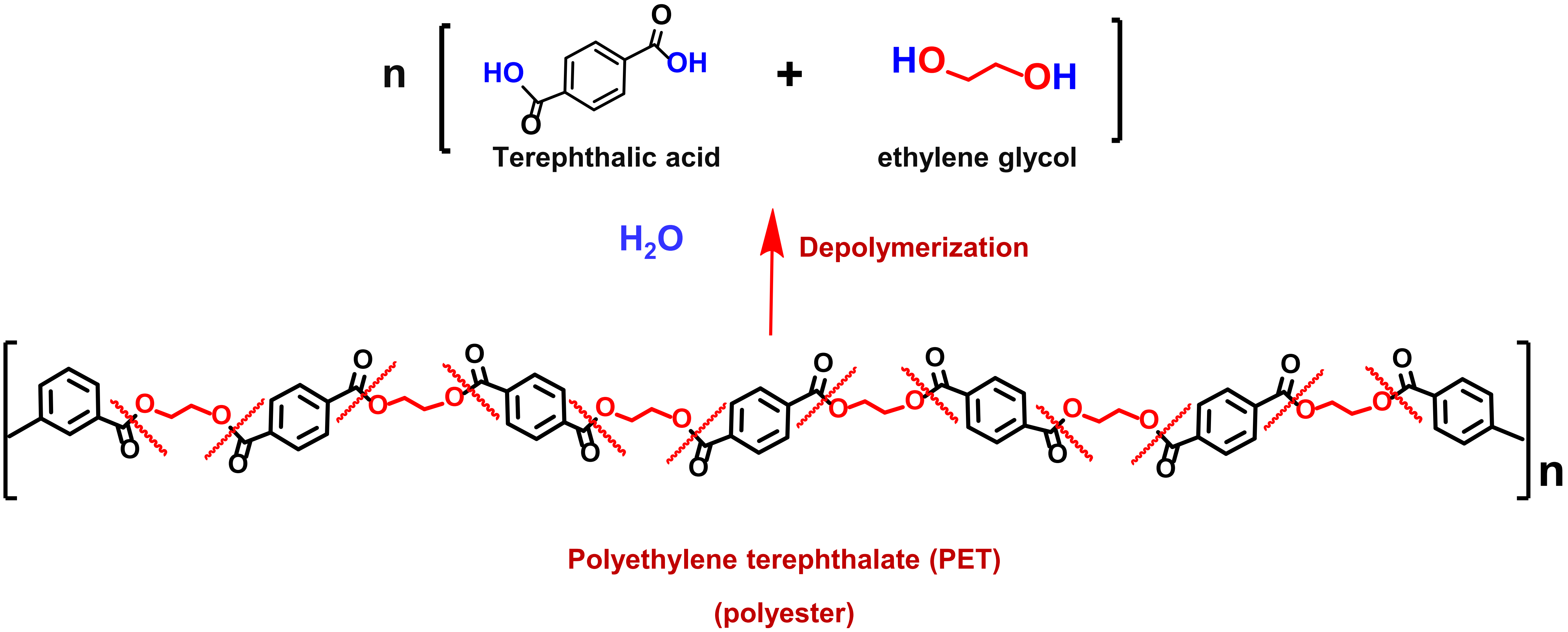
PET POLYMERIZATION



Polyethylene terephthalate (PET)

(polyester)

PET DEPOLYMERIZATION



Biotech Center
July 22, 2024

USES AND TOXICITY OF END PRODUCTS (RAW MATERIALS)

Terephthalic acid:

- Reused as raw material for plastic production leading to **‘circular’** economy
- Pharmaceutical industry
- Component in metal-organic framework
- Filler in some military smoke grenades
- Toxicity: LD_{50} = 1000 mg/kg (oral, mouse)
  aspirin: LD_{50} = 250 mg/kg (oral, mouse)

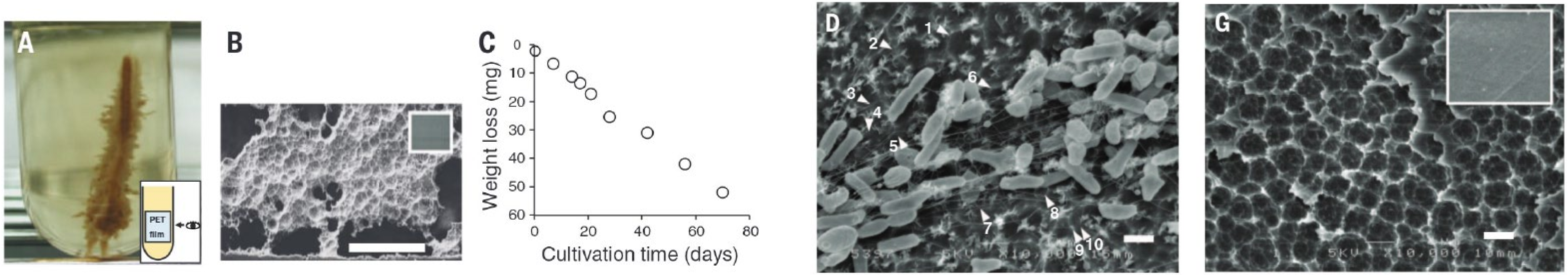
Ethylene glycol:

- Antifreeze, hydraulic fluid, paint solvent
- Toxicity: LD_{50} = 785 mg/kg (oral, human); 7712 mg/kg (oral, rat)
  ethanol: LD_{50} = 2000 mg/kg (oral, human); 7060 (9000) mg/kg (oral, rat)

BACTERIAL PET BIODEGRADATION

Novel bacterium: *Ideonella sakaiensis* 201-F6

- Pioneered by Yoshida, et al. in 2016
- Screened for microorganisms using low-crystallinity (1.9%) PET film as the carbon source
- Isolated a novel bacterium from a collection of 250 PET debris-contaminated environmental samples: sediment, soil, wastewater, and sludge from a PET recycling plant (for 5 yrs, Osaka)
- Bacterium: used PET as the major energy and carbon source
- Shares 51% AA sequence of the catalytic residues with a hydrolase from *Thermobifida fusca*



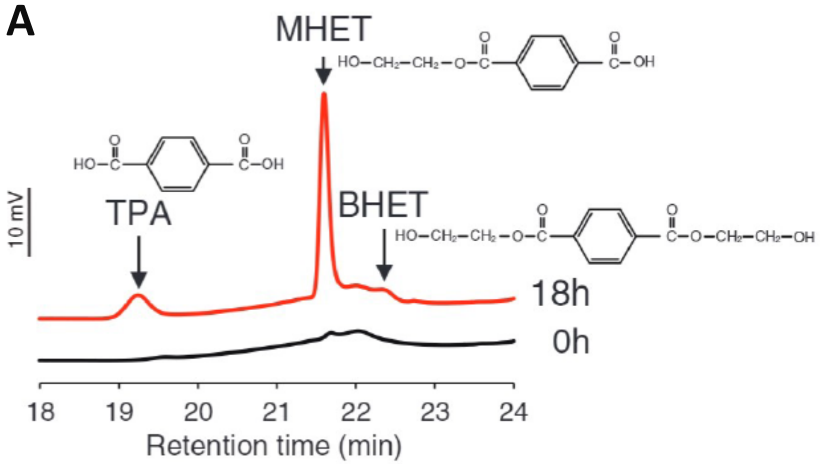
(A) Growth of a consortium of bacteria, yeast-like cells and protozoa on PET film (60 mg, 20x15x0.2 mm) at 30^{o}C after 20 d; (B) SEM of PET film after 70 d; (C) PET degradation vs time; (D) SEM image of *I. sakaiensis* grown on PET film (60 hrs); and, (G) SEM image of PET film after washing out adherent cells. Inset shows intact PET film.

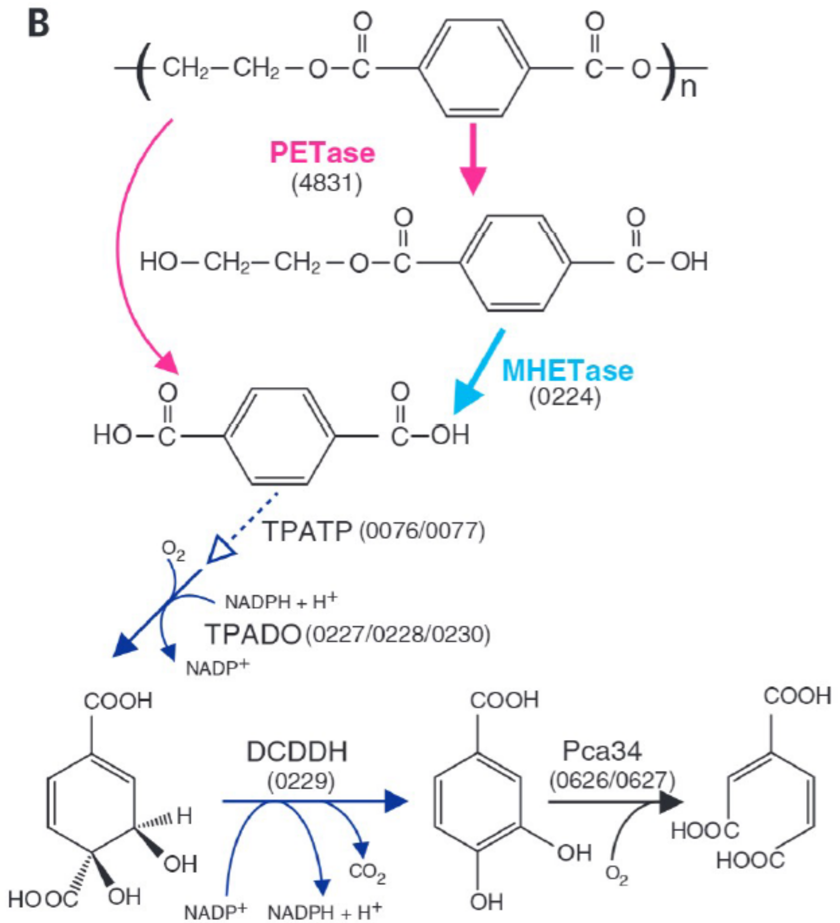
Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K. *A bacterium that degrades and assimilates poly(ethylene terephthalate)*. Science. 2016; 351(6278): 1196-9. Doi: 10.1126/science.aad6359.

BACTERIAL PET METABOLISM WITH PETase

Novel bacterium: *Ideonella sakaiensis* 201-F6

- PETase: PET digesting enzyme isolated
- End products: terephthalic acid (TPA), mono(2-hydroxyethyl) terephthalate (MHET) and bis(2-hydroxy ethyl) terephthalate (BHET)





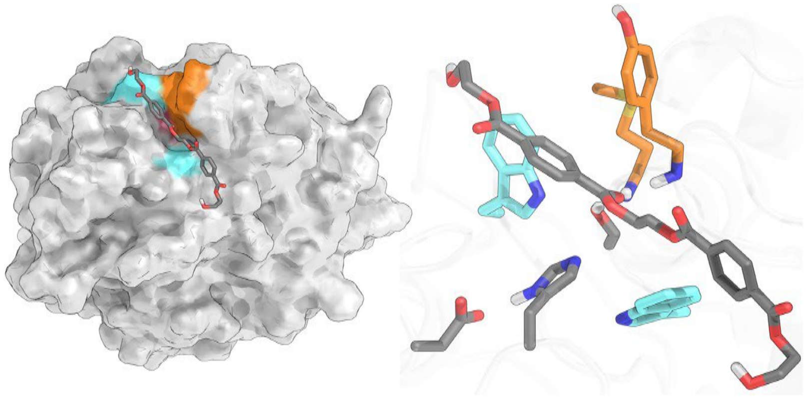
(A) High performance liquid chromatography (HPLC) spectrum of the products released from the PET film; (B) TPA is incorporated into TPA transporter, catabolized by TPA 1,2-dioxygenase, then by dihydroxy-3,5-cylohexadiene-1,4-dicarboxylate dehydrogenase, followed by ring opening with PCA 3,4-dioxygenase producing 3-carboxyl-hexa-2,4-diene-1,6-dioic acid.

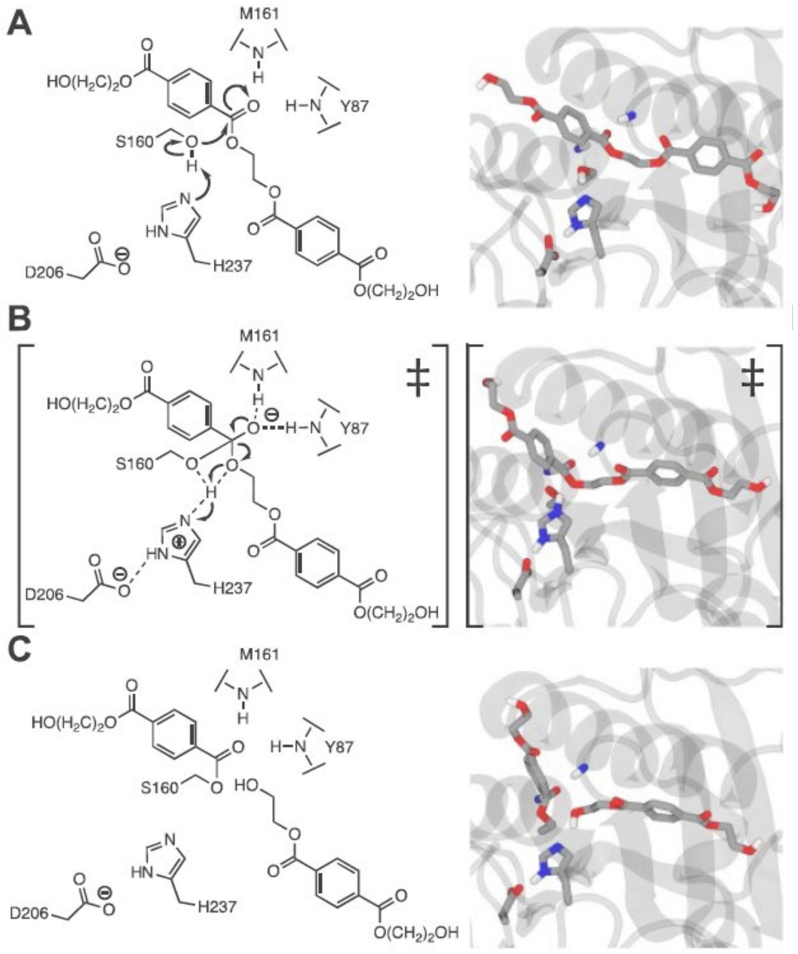
Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K. *A bacterium that degrades and assimilates poly(ethylene terephthalate)*. Science. 2016; 351(6278): 1196-9. Doi: 10.1126/science.aad6359.

PETase COMPUTER SIMULATION

PETase acylation reaction:

- Surface depiction of the equilibrated Michaelis complex: PETase (PDB code: 6EQE) with PET dimer
- Unbiased quantum mechanical and molecular mechanical (QM/MM) molecular dynamics (MD) simulation





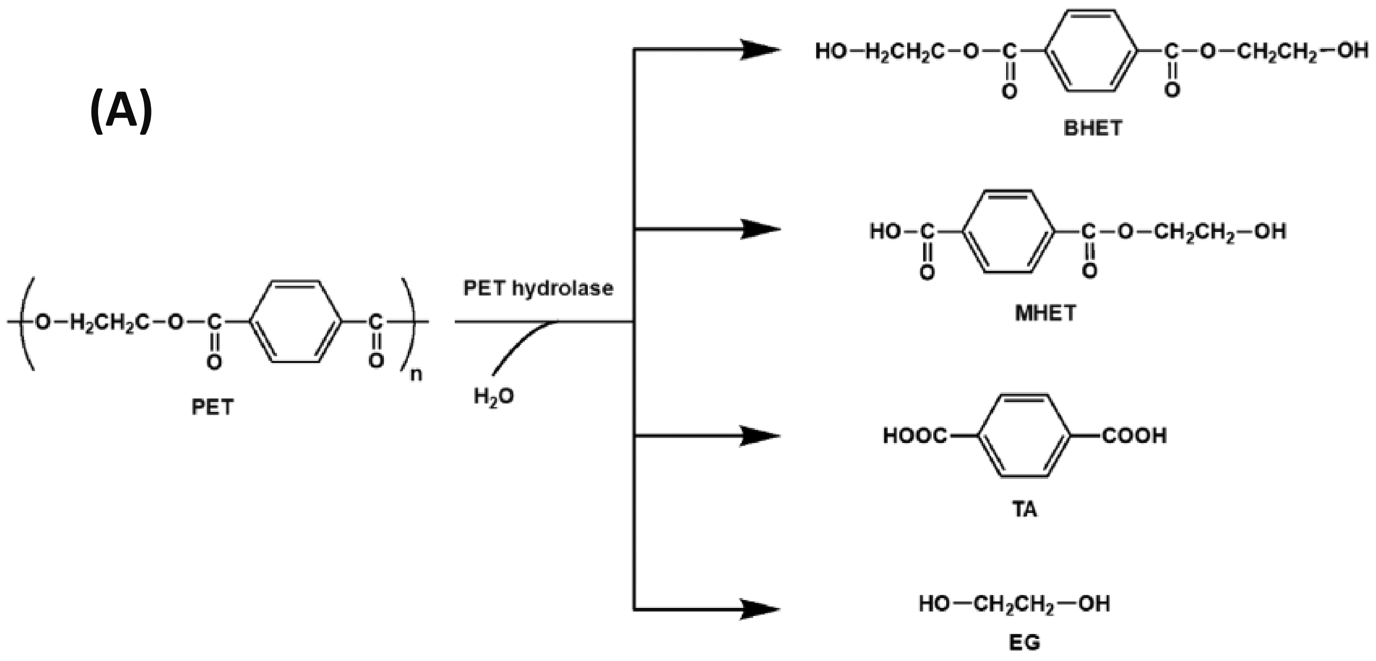
Interaction via H-bonds between the catalytic residues and PET carboxyl group and the oxyanion hole. Catalytic triad (serine, histidine, and aspartate) with the oxyanion hole at the active site of PETase interacting with PET dimer; (B) Transition state (TS) showing nucleophilic attack of hydroxyl group of serine, with its hydrogen being transferred to histidine; (C) Acyl enzyme intermediate.

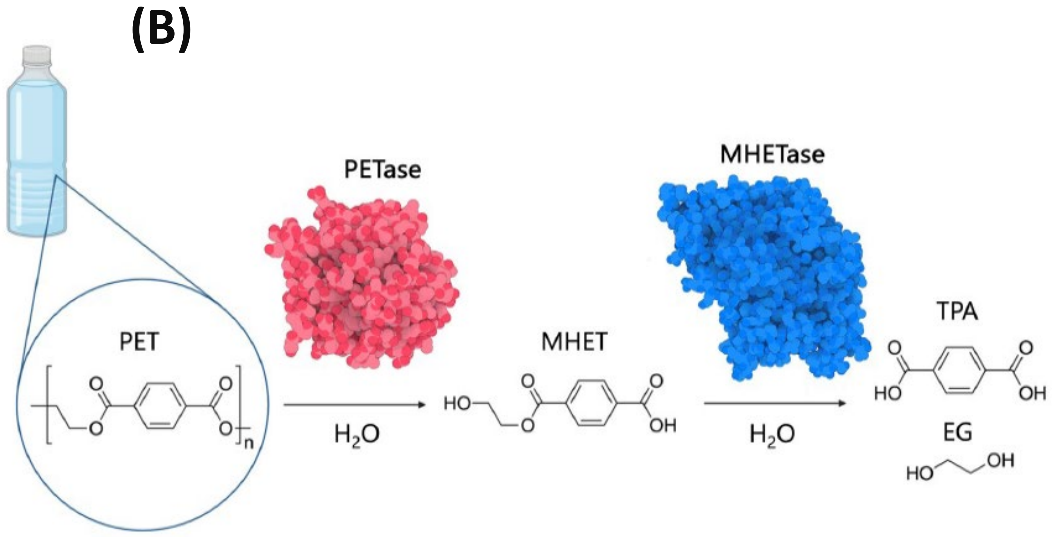
Burgin T, Pollard BC, Knott BC, Mayes HB, Crowley MF, McGeehan JE, Beckham GT, Woodcock HL. *The reaction mechanism of the Ideonella sakaiensis PETase enzyme*. Commun. Chem. 2024; 7, 65. Doi: 10.1038/s42004-024-01154-x; Barclay A, Acharya KR. *Engineering Plastic Eating Enzymes Using Structural Biology*. Biomolecules. 2023; 13(9): 1407. Doi: 10.3390/biom13091407;

PETase REACTION PRODUCTS

PETase acylation reaction:

- 4 End products





(A) PET and hydrolyzed products; (B) Sequential action of PETase and MHETase isolated from *I. sakaiensis*, that degrade PET into 4 products. PDB codes 6EQD for PETase and 6QZ1 for MHETase.

Burgin T, Pollard BC, Knott BC, Mayes HB, Crowley MF, McGeehan JE, Beckham GT, Woodcock HL. *The reaction mechanism of the Ideonella sakaiensis PETase enzyme*. Commun. Chem. 2024; 7, 65. Doi: 10.1038/s42004-024-01154-x; Barclay A, Acharya KR. *Engineering Plastic Eating Enzymes Using Structural Biology*. Biomolecules. 2023; 13(9): 1407. Doi: 10.3390/biom13091407;

PLASTIC DEGRADING BACTERIA

Enzymes and optimum conditions for plastic degradation:

Table 1. Enzymes and degradation factors corresponding to degrading bacteria.

Source	Enzyme	Major Mechanism of Degradation	Plastics/ Mircoplastics	Optimum Conditions	References
Ideonella sakaiensis 201-F	PETase	Hydrolysis	PET	Temperature 70–75 °C	[35]
Ideonella sakaiensis 201-F	MHETase	Hydrolysis	PET	Temperature 70–75 °C	[35]
Amycolaptosis sp.	——	Hydrolysis	PLA	Temperature 50 °C	[54]
Aspergillus oryzae	——	Hydrolysis	PBS	Temperature 50 °C	[15]
Penicillium funiculosum	——	Hydrolysis	PHB	Temperature 50 °C	[15]
Thermomonospora and curvata(cutinase homolog from leaf-branch compost)	LC-cutinase	Hydrolysis	PET	Temperature 50 °C	[65]
Thermophilic, alkaliphilic, halophilic, and psychrophilic bacteria	Bacteriophilic enzyme	Hydrolysis	Various plastic	Salt, low, or high pH, temperatures	[67]
Pseudomonas, Arthrobacter	PME hydrolases	Hydrolysis and oxidation	PVC, PP, PE, PS (PAEs)	Temperature 30–70 °C	[13]
Bacillus sp. *GZB*	A spore-laccase	The expression of different functional genes	PC (BPA)	Adding electron donors and co-substrates	[69]
Aspergillus sp. *strain ST-01*	Catalase, Protease	Colonization	PCL	Temperature 50 °C	[56]

Cai Z, Li M, Zhu Z, Wang X, Huang Y, Li T, Gong H, Yan M. *Biological Degradation of Plastics and Microplastics: A Recent Perspective on Associated Mechanisms and Influencing Factors.* Microorg. 2023; 11(7):1661. Doi: 10.3390/microorganisms11071661.

FUNGAL PLASTIC BIODEGRADATION

Cutinases for PET:

- Esterases that can hydrolyze polyesters of high MW
- α/β Hydrolases present in plant pathogenic fungi: *Fusarium solani*
  Humicola insolens
  Aspergillus oryza
  Candida antarctica
  Penicillium citrinum

Laccases for Polyethylene and Polystyrene:

- Oxidases; break phenolic compounds
- Edible fungi/mushrooms: *Pleurotus abalones*
  Pleurotus ostreatus
  Agaricus bisporus

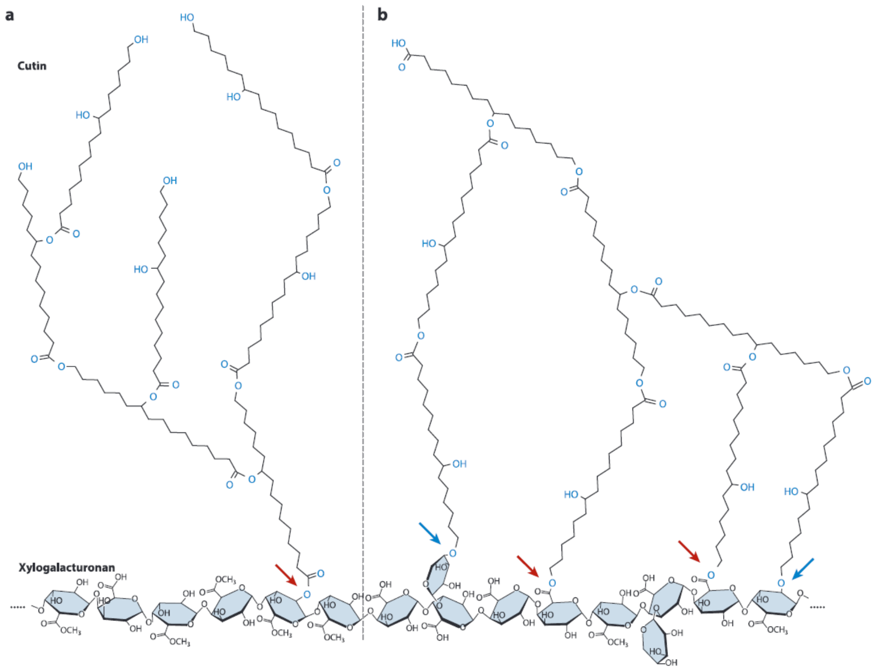
Note: very few fungi shows effective biodegradation

Srikanth M, Sandeep TSRS, Sucharitha K, Godi S. *Biodegradation of plastic polymers by fungi: a brief review*. Bioresour. Bioprocess. 2022; 9(1): 42. Doi: 10.1186/s40643-022-00532-4; Sooksai T, Bankeeree W, Sangwatanaroj U, Lotrakul P, Punnapayak H, Prasongsuk S. *Production of cutinase from Fusarium falciforme and its application for hydrophilicity improvement of polyethylene terephthalate fabric*. 3 Biotech. 2019; 9(11): 389. Doi: 10.1007/s13205-019-1931-1.

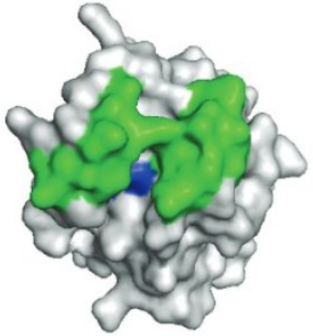
PLANT CUTIN DEPOLYMERIZATION

Cutin:

- Insoluble polyester of long chain hydroxy fatty acid
- Polymeric structure linked to polysaccharide
- Chemically-stable and resistant to decay
- Serves as framework of the plant cuticle
- First line of defense against pathogens (bacteria/fungi) to colonize aerial organs to secrete cutinases to depolymerize cutin
- Cutinase in *Fusarium solani* (1AGY) showing the active sites that are exposed and are sufficient to interact with the substrates



c *Fusarium solani* cutinase (1AGY)

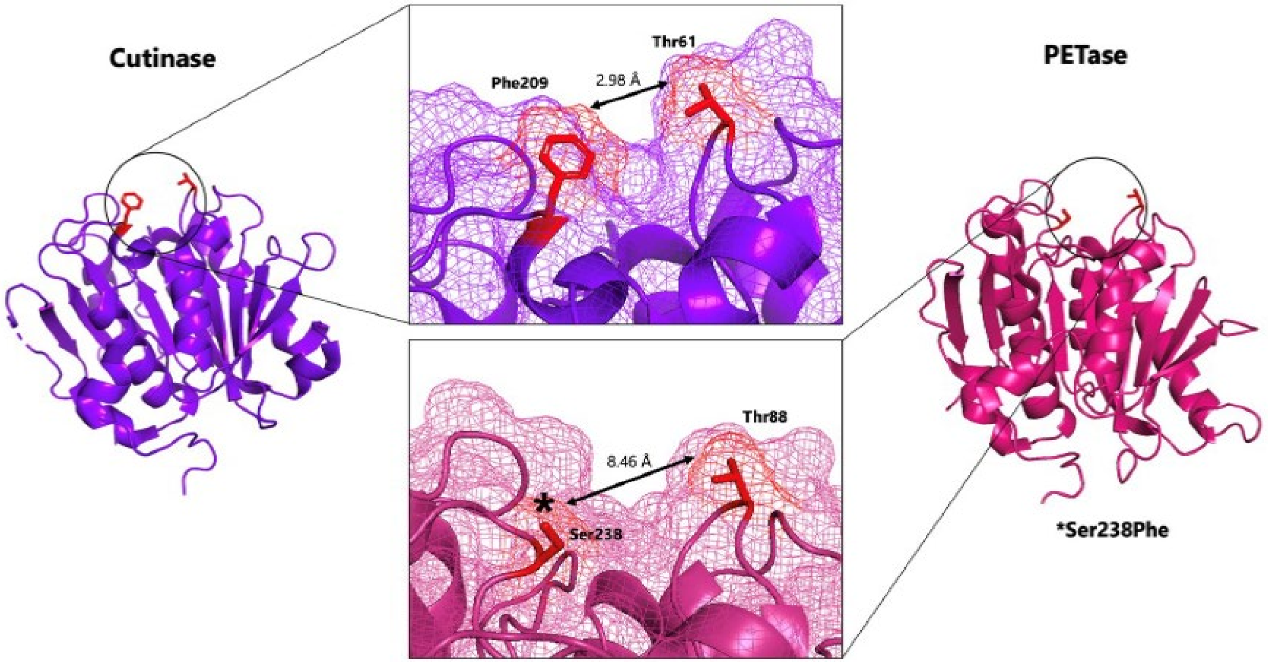


Fich EA, Segerson NA, Rose JKC. *The Plant Polyester Cutin: Biosynthesis, Structure, and Biological Roles*. Ann. Rev. Plant Biol. 2016; 67: 207-33. Doi: 10.1146/annurev-arplant-043015-111929.

CUTINASE MOLECULAR DYNAMICS SIMULATION

PETase vs Cutinase:

- Share considerable sequence homology; long substrate binding cleft (~40 Å)
- PDB codes: PETase (6EQD)
  Cutinase (4CG1)
- Major difference: Widening of the substrate binding cleft in PETase compared to cutinase
- Evolutionary adaptation to accommodate the semi-aromatic and more crystalline PET substrate
- Created a mutant *Ser238Phe, made the cleft narrow but increased enzyme activity
- Protein engineering to enhance PETase activity



Austin HP, Allen MD, Donohoe BS, Rorrer NA, Kearns FL, Silveira RL, Pollard BC, Dominick G, Duman R, El Omari K, Mykhaylyk V, Wagner A, Michener WE, Amore A, Skaf MS, Crowley MF, Thorne AW, Johnson CW, Woodcock HL, McGeehan JE, Beckham GT. *Characterization and engineering of a plastic-degrading aromatic polyesterase*. Proc. Natl. Acad. Sci. USA. 2018; 115(19): E4350-E4357. Doi: 10.1073/pnas.1718804115.

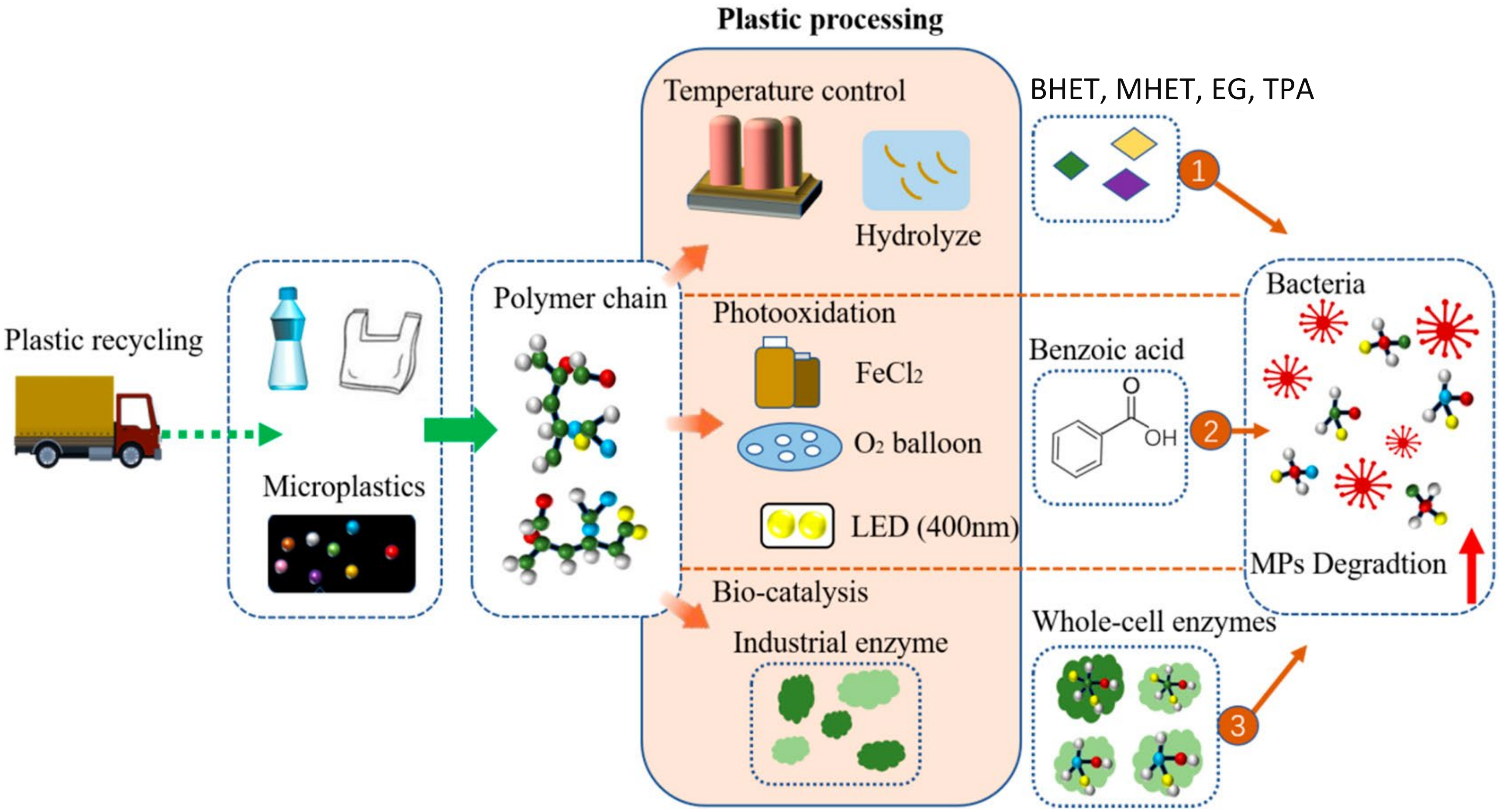
PLASTIC-EATING ENZYMES

Type of plastic	Degrading enzymes
Low-density polyethylene	Cutinase, laccase, and peroxidase
Poly–Ɛ-caprolactone	Cutinase, lipase, and PETase
Poly(1,4-butylene 2,5-furandicarboxylate)	Cutinase
Polybutylene succinate	Cutinase, PHB-depolymerase, and lipase
Polyethylene succinate	PHB-depolymerase
PET	Cutinase, and PETase
Polyhydroxybutyrate	Cutinase, and PHB-depolymerase
Polylactic acid	Cutinase, esterase, lipase, protease, and proteinase K
Polyurethane	Cutinase
Polyvinyl chloride	Laccase, and peroxidase

Patel SKS, Lee JK. *Plastic Eating Enzymes: A Step Towards Sustainability*. Indian J. Microbiol. 2022; 62(4): 658-661. Doi: 10.1007/s12088-022-01041-w.

PLASTIC WASTE PRETREATMENT

- Enhance plastic and microplastic biodegradation by physical and chemical means



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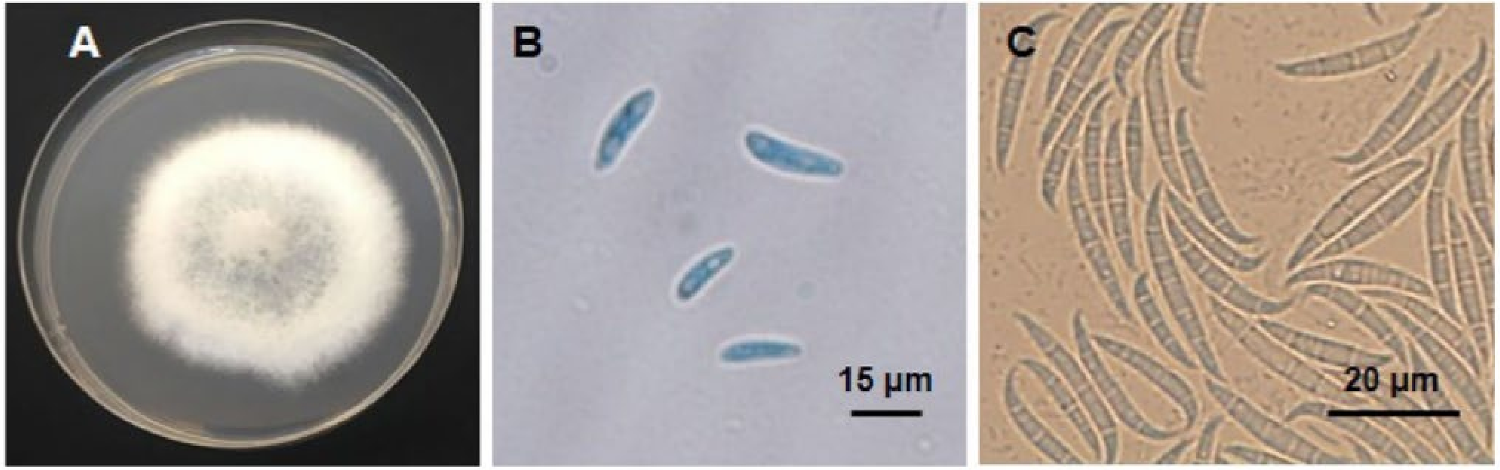
ENZYMATIC PET FABRIC PRETREATMENT

Cutinase for PET:

- Identified cutinase-producing fungi *Fusarium falciforme*
- Modified surface of PET fabric (wettability, fabric softness) by changing PET hydrophilicity
- Better than the conventional alkali (NaOH) treatment
- Cutin from papaya peel (cucumber peel, eggplant peel, pomelo field soils, etc.)
- Caveat: can cause wilt disease



Sooksai T, Bankeeree W, Sangwatanaroj U, Lotrakul P, Punnapayak H, Prasongsuk S. *Production of cutinase from Fusarium falciforme and its application for hydrophilicity improvement of polyethylene terephthalate fabric*. 3 Biotech. 2019; 9(11): 389. Doi: 10.1007/s13205-019-1931-1; Thao LD, Anh PT, Trang TTT, Khanh TN, Hien LT, Binh VTP, Thanh HM, Thanh DT. Fusarium falciforme, a pathogen causing wilt disease of chrysanthemum in Vietnam. New Dis. Rep. 2021; 43:e12013. Doi: 10.1111/ndr2.212013.

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