A new approach to oceanic PET decomposition using halophilic microorganisms and salt-tolerant enzymes

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Research objective

Plastic pollution in marine environments has become a global concern in recent years. Plastics discharged into the ocean at a density higher than that of seawater sink immediately and therefore accumulate on the seafloor. In contrast, low-density plastics like such as polyethylene terephthalate (PET) float on the ocean surface before washing ashore as salt-contaminated marine debris. These plastics are then degraded by ultraviolet radiation to become microplastics, which eventually settle in the ocean. Once collected, beached plastic debris is typically disposed in landfills or incinerated. However, landfilling can release high concentrations of chloride ions into public water systems due to adhered salt. Incineration produces hydrogen chloride gas, accelerating incinerator deterioration.

In 2016, a PET-degrading enzyme (PETase) was discovered from *Ideonella sakaiensis* strain 201-F6[1)], raising expectations for biological recycling. PETase hydrolyzes PET to mono(2-hydroxyethyl) terephthalate (MHET) (Fig. 1). *I. sakaiensis* possesses MHETase, which degrades MHET, enabling complete PET degradation through both enzymes[2)]. However, salt-contaminated marine debris contains high salt concentrations, making non-halotolerant PETase ineffective. Furthermore, PETase exhibits low thermal stability at an optimal reaction temperature of 40 °C, limiting industrial applications.

In this study, we aimed to develop novel halotolerant and salt-resistant PETases for degrading salt-contaminated plastic debris and evaluate their PET hydrolysis ability under such conditions.

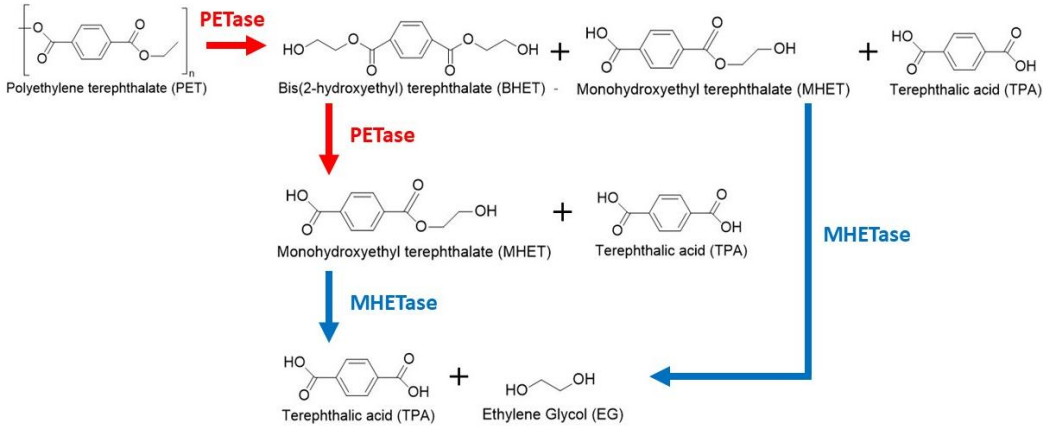

Fig. 1 PETase and MHETase-mediated PET decomposition

Methods

Wild-type and mutant PETases were prepared by transforming *Escherichia coli* with

plasmids encoding either wild-type or mutant genes, followed by the purification of recombinant enzymes from the transformants. The enzymatic activity of recombinant PETase was evaluated by incubating the enzyme with a substrate (*p*-nitrophenyl butyrate) at pH 7.0 and various salt (NaCl) concentrations (0–5.0 M) for 30 s at 30 °C, and measuring absorbance at 415 nm. The solvent-accessible surface area of wild-type PETase was calculated using a protein modeling software.

Results

1. Characterization of wild-type PETase

   To investigate wild-type PETase activity in the presence of salt, the purified enzyme was reacted with the substrate at varying salt concentrations (0–5.0 M), and its activity was measured. The results showed that wild-type PETase exhibited the highest activity at 0.5 M NaCl. Although the enzyme retained moderate activity up to 3.0 M NaCl, a sharp decline in activity was observed at concentrations greater than 4.0 M.

   To further assess the response of PETase to salt stress, the stability of wild-type PETase was evaluated by incubating the enzyme at different NaCl concentrations (0–5.0 M), followed by activity measurements. The results indicated that enzymatic activity decreased progressively with increasing salt concentration, and at 5.0 M NaCl, the activity dropped to approximately 40% of its maximum. These findings suggest that wild-type PETase exhibits low halotolerance under high-salt conditions.

2. Development of novel salt-tolerant PETase

   To obtain a PETase that is active and stable under high-salinity conditions, two strategies were used to develop a novel salt-tolerant PETase: 1) engineering a salt-tolerant esterase with altered substrate specificity to create a PET-degrading enzyme and 2) enhancing the salt tolerance of PETase to generate a salt-tolerant variant.

1) Creation of a salt-tolerant pet-degrading enzyme via substrate specificity modification of esterase

   Plastics are polymers formed through ester bonds between aliphatic chains. Accordingly, PET-degrading enzymes likely evolved from lipases or esterases, and several lipases and esterases capable of degrading plastics have been reported[3]. Our laboratory has previously isolated and characterized a salt-tolerant esterase from *Haloarcula japonica*, an extremely halophilic archaeon[4]. The plastic-assimilating ability of *H. japonica* was investigated for its potential PET degradation. The results showed that this organism cannot grow using plastic as its sole carbon source. Therefore, mutations have been introduced into salt-tolerant esterases to develop PET-degrading enzymes with enhanced salt tolerance. A screening system for mutant PETase variants is currently under development.

2) Enhancing the salt tolerance of PETase itself to generate a salt-tolerant variant

To generate a salt-tolerant PETase, amino acid substitutions were introduced into the recombinant PETase to produce mutant variants. Previous studies showed that salt-tolerant proteins derived from halophilic microorganisms typically possess a high abundance of acidic amino acids and low content of basic amino acids on their molecular surfaces[5]. Based on this, acidic amino acids were introduced and basic amino acids were removed from the molecular surface of PETase to enhance salt tolerance. The solvent accessibility of each amino acid residue was calculated, and highly exposed residues were substituted with aspartic acid to create multiple mutant enzymes. To evaluate the enzymatic activity of these PETase variants under saline conditions, reactions were conducted with the substrate at various NaCl concentrations (0–5.0 M). One mutant enzyme, where the 32nd arginine residue was replaced with aspartic acid, exhibited the highest activity at 3.0 M NaCl and maintained greater activity than the wild-type enzyme under high-salinity conditions (4.0 and 5.0 M NaCl).

Conclusion

Efforts have been made to develop a salt-tolerant PETase capable of degrading salt-contaminated plastic waste. Site-directed mutagenesis was applied to a previously reported PETase, resulting in mutant enzymes with optimal salt concentrations and enhanced activity under high-salinity conditions. Future studies should evaluate the activity of mutant PETases against PET films in the presence of salt. This study demonstrated that site-specific amino acid substitutions can yield salt-tolerant PETase variants. The application of such enzymes offers promising potential for degrading salt-contaminated plastic waste.

References

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