Noda Institute for Scientific Research GRANT
2012 Young Investigator Research Grant

Metabolic and enzymatic analyses of a poly(ethylene terephthalate) assimilation bacterium

Shosuke YOSHIDA

Department of Biosciences and Informatics, Keio University

Research aims

Poly(ethylene terephthalate) (PET) is one of the most commonly used plastic materials because it is chemically inert and non-biodegradable. The Oda group (Kyoto Institute of Technology) screened for PET-degrading bacteria from the natural environment and successfully isolated a PET-assimilating bacterium *Ideonella* sp. No. 201-F6 strain. This discovery allowed us to identify novel enzymes from the bacterium that degrade PET, an unnatural material. The aim of this study was to use genomic information of the No. 201-F6 strain, which we ascertained with next-generation sequencing, to understand the unique enzymatic scheme that allows PET to be used as a sole carbon source.

Methods

(1) *Whole genome analysis of Ideonella sp. No. 201-F6 strain*

After culturing No. 201-F6 strain cells in a nutrient-rich medium, genomic DNA was extracted, fragmented with an Acoustic Solubilizer (Covaris Inc.), and converted to a library for next-generation sequencing by using a Paired-End Sample Preparation Kit (Illumina Inc.). Sequencing with a read length of 80 nt was performed using the Genome Analyzer IIx (Illumina Inc.). The reads were assembled into contigs by using CLC Genomics Workbench (CLC bio Inc.). Genes were annotated on the Rapid Annotation using Subsystems Technology (RAST) server.

(2) *Discovery of PET-hydrolyzing enzyme*

The annotated putative genes of the No. 201-F6 strain were searched in silico for hydrolases such as esterases and lipases. Similarly, the sequence data were searched for homologs of cutinases, which have been reported as PET-hydrolyzing enzymes.[1,2,3)]

(3) *Functional analysis of the ORF2645 protein*

The codons of ORF2645 gene were optimized for expression in *Escherichia coli*, and the whole gene was synthesized. The optimized gene was ligated into the pET-21b(+) vector. Proteins were expressed in BL21-CodonPlus(DE3)-RIPL (Agilent Inc.). Proteins were purified by nickel-affinity chromatography. The activity of purified proteins was evaluated using a PET film, bis(2-hydroxyethyl)terephthalic acid (BHET), and *para*-nitorophenyl (*p*NP)-butyrate.

Results

(1) *Whole genome analysis of Ideonella sp. No. 201-F6 strain*

Fifty-one million reads with an average length of 74.9 nt were obtained by sequencing. Denovo assembly analysis constructed 2892 contigs, ranging from 93,997 nt to 187 nt, from the single reads. The RAST server annotated 3960 putative genes from the contigs.

(2) *Discovery of PET-hydrolyzing enzyme*

The genes of 42 putative esterases and 8 putative lipases were found in the annotated No. 201-F6 genes. One of the putative lipases, ORF2645 protein, showed 51% identity with a cutinase from *Thermobifida fusca* and 82% identity with PBSA (Poly{(tetramethylene succinate)-co-adipate}) depolymerase from *Acidovorax delafieldii*.[4)]

(3) *Functional analysis of ORF2645 protein*

Crater-shaped pits were formed on the surface of PET film by the activity of purified ORF2645 protein (Fig. 1). This protein also is enzymatically active on BHET, and produced mono(2-hydroxyethyl) terephthalic acid with a turnover rate of 6.3 s^{-1}. The turnover rate for hydrolysis of *p*NP-butyrate by this enzyme was 30 s^{-1}.

Conclusion

In this study, we successfully identified a novel PET hydrolase, ORF2645 protein, from a PET-assimilating bacterium *Ideonella* sp. No. 201-F6 strain. Detailed biochemical characterization of this protein is ongoing. By using RNA-Seq analysis, we are attempting to identify additional No. 201-F6 proteins that may be involved in PET degradation.

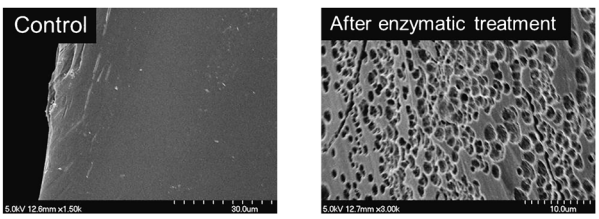

Fig. 1. Enzymatic degradation of PET film surface by ORF2645 protien (SEM images)

References

1) Sulaiman, S., Yamato, S., Kanaya, E., Kim, J.-J., Koga, Y., Takano, K., and Kanaya, S. (2012) Isolation of a novel cutinase homolog with polyethylene terephthalate-degrading activity from leaf-branch compost by using a metagenomic approach. *Appl. Environ. Microbiol.* **78**: 1556–62.
2) Müller, R.-J., Schrader, H., Profe, J., Dresler, K., and Deckwer, W.-D. (2005) Enzymatic Degradation of Poly(ethylene terephthalate): Rapid hydrolyse using a hydrolase from *T. fusca*. *Macromol. Rapid Commun.* **26**: 1400–5.
3) Eberl, A., Heumann, S., Brückner, T., Araujo, R., Cavaco-Paulo, A., Kaufmann, F., Kroutil, W., and Guebitz, G. M. (2009) Enzymatic surface hydrolysis of poly(ethylene terephthalate) and bis(benzoyloxyethyl) terephthalate by lipase and cutinase in the presence of surface active molecules. *J. Biotechnol.* **143**: 207–12.
4) Uchida, H., Shigeno-Akutsu, Y., Nomura, N., Nakahara, T., Nakajima-Kambe, T. (2002) Cloning and sequence analysis of poly (tetramethylene succinate) depolymerase from *Acidovorax delafieldii* strain BS-3. *J. Biosci. Bioeng.* **93**: 245–247.