Biodegradation of different PET variants from food containers by *Ideonella sakaiensis*

Andreas Walter¹ · Laura Sopracolle¹ · Mira Mutschlechner¹ · Martin Spruck² · Christoph Griesbeck¹

Received: 16 August 2022 / Revised: 29 September 2022 / Accepted: 26 October 2022 / Published online: 16 November 2022
© The Author(s) 2022

Abstract

The accumulation of macro-, micro- and nano-plastic wastes in the environment is a major global concern, as these materials are resilient to degradation processes. However, microorganisms have evolved their own biological means to metabolize these petroleum-derived polymers, e.g., *Ideonella sakaiensis* has recently been found to be capable of utilizing polyethylene terephthalate (PET) as its sole carbon source. This study aims to prove its potential capacity to biodegrade two commercial PET materials, obtained from food packaging containers. Plastic pieces of different crystallinity were simultaneously introduced to *Ideonella sakaiensis* during a seven-week lasting investigation. Loss in weight, appearance of plastics, as well as growth of *Ideonella sakaiensis*—through quantitative real-time PCR—were determined. Both plastics were found enzymatically attacked in a two-stage degradation process, reaching biodegradation capacities of up to 96%. Interestingly, the transparent, high crystallinity PET was almost fully degraded first, followed by the colored low-crystallinity PET. Results of quantitative real-time PCR-based gene copy numbers were found in line with experimental results, thus underlining its potential of this method to be applied in future studies with *Ideonella sakaiensis*.

Keywords *Ideonella sakaiensis* · Polyethylene terephthalate · qPCR · Waste management · Recycling · Micro-plastic

Introduction

Since its introduction in 1941, polyethylene terephthalate (PET) has become one of the most extensively applied polymers with a worldwide production of more than 400 million tons in 2020 (Skoczinski et al. 2021), predominantly used for the production of synthetic fibers and single-use plastic bottles and food containers (Wallace et al. 2020). Although PET is recyclable, high percentages of plastic waste accumulate in the ecosystems, causing a severe environmental burden, which is mostly undiscovered (Wallace et al. 2020; Prata et al. 2019; Chen et al. 2018). Hence, plastic degradation is one of the major global challenges, which have to be tackled urgently (Aer et al. 2022).

In 2016, a novel bacterium was detected, which may be advantageous to remove PET from the environment (Yoshida et al. 2016; Tanasupawat et al. 2016). Sampled in a PET recycling side in Sakai city, Japan, *Ideonella sakaiensis* (ISAK) is capable to utilize PET as its sole carbon source (Tanasupawat et al. 2016; Bornscheuer 2016). This Gram-negative strain reveals optimal growth rates at pH 7–7.5 and temperatures of 30–37 °C (Tanasupawat et al. 2016). Two key enzymes could be identified, both facilitating the degradation and assimilation of PET: the exo-enzyme PETase converts PET to mono-(2-hydroxyethyl) terephthalate (MHET) with a relatively high activity at room temperature, when compared to other PET-degrading enzymes (Aer et al. 2022). Then, in the cell periplasm, MHETase hydrolyzes MHET to the PET educts ethylene glycol (EG) and terephthalic acid (TPA), which is subsequently transported into the cytoplasm and introduced into the tricarboxylic acid cycle (Palm et al. 2019).

The aim of this study was to investigate the simultaneous biodegradation of two commercial PET-packing materials with different crystallinities—one colored PET (PETcol) and...
one transparent PET (PETtra), obtained from commercial food containers—by ISAK during an investigation period extending over 7 weeks in total. The proof of biodegradation capacities of commercial packaging materials, which were rarely investigated to date, will facilitate to understand the full potential of microorganisms and their enzymatic toolbox when it comes to the application of polymer recycling.

Materials and methods

Cultivation, media preparation and PET characterization

A pure culture of ISAK was obtained from the Biological Resource Centre, NITE (NBRC; Kazusakamatari, Japan) and cultivated in medium 802 (Wako Pure Chemical Industries, Ltd, Japan), according to NBRC online catalog. Growth temperature was set to 33.5 °C using a rotary shaker (150 rpm). Overnight cultures were cryo-conserved in 10% glycerine [v/v] at —80 °C to be used on demand in experiments.

Round PET pieces (Ø 6 mm), cut out from the sidewall of PETcol and PETtra with a hand-puncher were characterized (Table 1): all pieces were weighted on a MX5-Microbalance (Mettler Tole-do, Columbus, USA). Thickness was determined with an outside micro-meter (Digimatic Micrometer 0–25 mmm, Mitutoyo, Kawasaki, Japan). The crystallinity of both plastics was characterized by differential scanning calorimetry (DSC, Mettler DCS 12 E, Mettler Toledo, Switzerland). PET pieces (n = 3) were heated in a nitrogen atmosphere at a heating/cooling rate of 10 °C min⁻¹. The enthalpies of the crystallization peak (Hc), and melt enthalpies (Hm) revealed values of 21.3 and 17.8 Jg⁻¹ and 37.7 and 21.9 Jg⁻¹, for PETtra and PETcol, respectively. The crystallinity (%) was calculated using the equation (Hm—Hc)/Hm100 × 100 (Table 1).

Degradation experiment

The degradation trials were conducted in shaking flasks containing 50 ml yeast extract–sodium carbonate–vitamins (YSV) medium, according to Tanasupawat et al. (2016). All PET pieces were sterilized in 70% ethanol for 48 h before application. Cell densities of 5.91 × 10⁴ (± 1.68 × 10³) CFU ml⁻¹, obtained from ISAK cryo-cultures growing in medium 802 for 21.5 h, were used to inoculate treatments. Both PET materials were treated simultaneously in the same shaking flasks. Growth temperature was kept constant at 33.5 °C on a rotary shaker (150 rpm). Three shaking flasks were removed weekly to determine physicochemical and biological analyses while the rest of the flasks remained incubated. After 7 weeks, the last three treatments, as well as the negative controls (n = 3)—containing YSV and PET, without ISAK—and the positive controls (n = 3)—inoculated with ISAK in YSV, without PET—were removed to terminate the degradation experiment.

Physicochemical parameters

PET pieces were removed through a sieve (0.5 mm mesh size; Karl Weis, Murr, Germany), carefully washed in ethanol (70%) and Aqua dest and dried at room temperature overnight prior to weight determination thereafter. Microscopic changes of PET pieces were documented, using a stereo microscope (Krüss, Hamburg, Germany) at a magnification of 15× in combination with a Canon EOS 600D (Canon, Tokio, Japan). The pH was measured with a pH 3110 device (WTW, Weilheim, Germany) in the broth, immediately after PET pieces were removed.

To provide information on the surface microstructure, PET samples of weeks one, three, five and seven were analyzed using a JSM-IT200LA scanning electron microscope (SEM; JEOL, Tokyo, Japan) operated at 10 kV and supplied with a SE detector and a BSE detector. For sample preparation, PET pieces were sputtered with gold under an argon atmosphere, at a distance of 40 mm with a Sputter Coater 108auto (Cressington, Watford, UK).

Quantitative real-time application

Samples were collected in triplicates after each week and stored frozen until further processing (the rest of the flasks remained incubated as stated before). The samples were filtered through a 0.45 µm membrane (Whatman, UK), washed and cut into pieces with a sterile scalpel prior to extraction. DNA was extracted using the NucleoSpin® Microbial DNA Kit (Macherey–Nagel, Düren, Germany) according to the manufacturer’s instructions with proteinase K. Subsequently, the quantity and purity of the extracted DNA were evaluated via UV/VIS spectrophotometry with NanoDrop 2000cTM. Quantitative real-time PCR (qPCR) was performed on a CFX96 Touch Deep Well Real-Time PCR System (Bio–Rad, USA), with the primer pairs BAC338f and BAC805r (Yu

| Table 1 Characteristics of colored PET (PETcol) and transparent (PETtra) |
|----------------|----------------|----------------|
| PETcol          | PETtra          |                |
| Origin          | Cookie container* | Grape container* |
| Color           | Brown           | Transparent    |
| Thickness (µm)  | 182 (± 0.47)    | 167 (± 2.29)   |
| Weight (mg)     | 6.38 (± 0.46)   | 6.43 (± 0.58)  |
| Crystallinity (%) | 3.2 (± 1.93)  | 12.6 (± 1.35)  |

*Pictures of containers are presented in Fig. 1.
et al. 2005) being applied. Prior to amplification, the samples were subjected to an initial denaturation step at 95 °C for 5 min. Each run included non-template controls (NTC, UltraPure DNase/RNase-Free Distilled Water, Invitrogen, USA) as well as DNA extracts from positive controls inoculated with ISAK but without any PET material and negative controls without inoculation \((n = 3)\). After quantification, PCR products were checked via melting curve analysis. For construction of calibration curves, we used a genomic DNA standard from *Ideonella azotifigens* (DSM 21438) purchased from the German collection of microorganisms and cell cultures (DSMZ, Germany). Cycling conditions for quantification of the assays targeting bacterial 16S rRNA gene copies were as followed: 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s (35 cycles).

**Results and discussion**

In this study, the potential of ISAK to biodegrade two PET-packaging materials—one PETcol and one PETtra—obtained from commercial food containers was investigated in a 7-week lasing experiment. Appearance of PET, noticed by eye, changed within days, revealing opacity in PETtra and color change in PETcol (Fig. 1). Such observations were also noticed by Wallace et al. (2020), where amorphous PET, when treated with ISAK, became obviously cloudy within days. The PET surfaces became rougher and were found perforated after three and 7 weeks, for PETtra and PETcol, respectively. After 4 weeks, PETtra was found almost degraded, however revealing a residual ring of compressed PET (Suppl. Figure 1), due to cutting out PET pieces from plastic containers.

SEM observation (Fig. 2) confirmed enzymatic activity, causing extensive biodegradation on the smooth surface of initial PETcol (a) and PETtra (f). Pits and cavities were increasingly observed over time, revealing average diameters of 2.6 µm (± 0.71 µm), 3.44 µm (± 0.8 µm), 21.7 µm (± 9.99 µm) and 45.4 µm (± 9.08 µm) over time for PETcol (b–e), as well as 9.24 µm (± 3.19 µm) and 32.1 µm (± 4.39 µm) for PETtra, in the second and third week (g, h), respectively. Similar results in respect to plastic deconstruction were previously observed for gut microorganisms (Yang et al. 2014), hyper-thermophilic bacteria (Chen et al. 2020) and *Ideonella sakaiensis* (Yoshida et al. 2016).

The pH was found consistent throughout the overall experiment (7.03 ± 0.02), indicating suitable conditions for ISAK.

Degradation capacities for PETtra were found strongly accelerated in comparison to PETcol, as shown in Fig. 3, revealing losses of 13.5% (± 6.27%) and 7.84% (± 6.13%) after 1 week, 54.4% (± 0.35%) and 22.4% (± 3.04%) after 2 weeks, and 81.2% (± 8.4%) and 29.4% (± 2.02) after 3 weeks for PETtra and PETcol, respectively. After 4 weeks, PETtra biodegradation almost stopped, revealing stagnating biodegradation capacities of 94.7% (± 0.93%), 96.2% (± 0.97%), 95.9% (± 0.55%) and 96.8% (± 0.18%) over the last 3 weeks. Biodegradation of PETcol remained gradually, revealing an endpoint capacity of 63.9%
Fig. 2  Biodegradation of initial PETcol (a) and PETtra (f) pieces under 500× magnification over time: PETcol after one (b), three (c), five (d) and seven weeks (e), respectively. PETtra after one (g) and three (h) weeks, respectively. Thereafter, PETtra was found destructed, revealing a residual ring of compressed PET (Suppl. Figure 1). The white bars are indicating 50 µm.

(±17.5%). Negative controls, investigated after 7 weeks were found unaltered, revealing a weight loss of 0.05%.

qPCR results were found in line with degradation capacities. All approaches showed higher copy numbers when compared with the positive controls—containing ISAK but no PET— with LOG10 abundances of 4.72 (±0.17). LOG10 abundances of 5.39 (±0.12) were detected after 1 week of degradation, thereafter increasing slightly until week four, when PETtra was almost degraded revealing LOG10 abundances of 4.91 (±0.3). Subsequently, copy numbers started to increase again, reaching 6.64 (±0.65) after 7 weeks, probably due to PETcol degradation.
Although assimilation of PET films through ISAK was previously described in 2017, where Yoshida et al. found low crystallinity PET films (60 mg; 20 × 15 × 0.2 mm; 1.9% crystallinity) biodegraded by 33%, after 3 weeks of incubation in YSV broth at 30 °C (Yoshida et al. 2016), degradation capacities of commercial packaging materials were rarely investigated to date. In 2020, Wallace et al. investigated the degradation of different parts of a commercial PET bottles and reported that the bacterium was not capable to grow from high crystalline PET, which is the majority of plastic in PET bottles, within a 2-week lasting experiment (Wallace et al. 2020). On the contrary, anamorph or low crystalline parts, such as finish, shoulder top and the base of the bottle, as well as solidified anamorph PET, formerly of higher crystallinity, could be degraded efficiently under the same conditions. Authors estimated that percentages of non-degradable parts of plastic bottles could range from 52 to 82%. PET bottles as well as polyester textiles usually reveal crystallinities of 30–40% when compared to other PET packaging materials, ranging from approximately 6–8% crystallinity (Kawai et al. 2014; Ronkvist et al. 2009). Interestingly, in this research, crystallinity was not affecting degradation efficiency overall. Although ISAK was found capable to tackle both materials, with lower-crystalline PETcol showing accelerated biodegradation, when introduced simultaneously with PETtra. Authors have already proposed other important factors, such as (i) hydrophobicity, (ii) surface topography, or (iii) molecular size of synthetic polymers are important factors affecting their biodegradability (Tokiwa and Calabia 2007; Webb et al. 2012; Wei and Zimmermann 2017). However, for this study, it remains unclear which PET characteristics effected enzymatic degradation in particular.

**Conclusion**

In this study, we investigated the biodegradation potential of two commercial PET materials through *Ideonella sakaiensis*. This bacterium was capable to almost degrade all PET from a grape container within 4 weeks and to reduce PET, obtained from a cookie container, by more than 50% within 7 weeks. It could be shown that both plastics were simultaneously exploited, however revealing different degradation rates, as expected. A qPCR using universal bacterial primers was shown to be in line with the physicochemical results obtained and this molecular–biological approach may be used in future studies as well to reveal DNA quantities of plastic-degrading microorganisms. While predicted natural lifetimes of PET range from 25 (Liu et al. 2019) to hundreds of years (Austin et al. 2018), biotechnological approaches, under sufficient conditions, could open novel grounds in industrial application, e.g., a complete biodegradation to face pollution or the recycling of the monomers TPA and/or EG (Taniguchi et al. 2019).
Big potentials bear the technologies of genetic engineering: (Genetic Engineered Ideonella Sakaiensis Bacteria: A Solution of the Legendary Plastic Waste Problem 2018) proposed to modify ISAK genes with Azotobacter sp. genes to boost its survival capability in habitats, such as soil and water. Authors have demonstrated that PETase could be successfully expressed in Chlamydomonas reinhardtii and Escherichia coli, respectively (Aer et al. 2022; Kim et al. 2020; Shi et al. 2021). Others (e.g., (Ma et al. 2018; Son et al. 2019; Meng et al. 2021)) have screened PETase mutants and variants to improve enzymatic performance as well as thermal stability. In addition, screenings for other, potent plastic-degrading microorganisms have to be continued, including (1) thermophilic microorganisms for industrial applications and (2) salt-tolerant microorganisms to face ocean pollution (Taniguchi et al. 2019). As proposed by Karunatillaka et al. (2020), there are a significant number of previously uncharacterized proteins that hold the capability of plastic biodegradation. Beside degradation, these enzymes hold potentials for bio-sensing applications of pollutants (Gul et al. 2021).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03306-w.

Acknowledgements We thank Rene Nußbaumer for analysing the crystallinity of packaging materials as well as Bianca Werndle and Marlene Weber for their support in cultivating Ideonella sakaiensis.

Author contributions Conceptualization: A.W.; methodology: A.W., L.S., M.M. and M.S.; investigation: L.S., M.M. and M.S.; resources: C.G.; writing—original draft preparation: A.W., M.M.; writing—review and editing: A.W., M.S. and C.G.; all authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by MCI Management Center Innsbruck – Internationale Hochschule GmbH. This project was funded by Die Tiroler Wissenschaftsförderung (TWF; project number: 16685/5-2019).

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. Springer

References

Aer L, Jiang Q, Gul I et al (2022) Overexpression and kinetic analysis of Ideonella sakaiensis PETase for polyethylene terephthalate (PET) degradation. Environ Res 212:113472

Austin HP, Allen MD, Donohoe BS et al (2018) Characterization and engineering of a plastic-degrading aromatic polyesterase. Proc Natl Acad Sci 115:E4350–E4357

Bornscheuer UT (2016) Feeding on plastic. Science 351:1154–1155

Chen C-C, Han X, Ko T-P et al (2018) Structural studies reveal the molecular mechanism of PETase. FEBS J 285:3717–3723

Chen Z, Zhao W, Xing R et al (2020) Enhanced in situ biodegradation of microplastics in sewage sludge using hyperthermophilic composting technology. J Hazard Mater 384:121271. https://doi.org/10.1016/j.jhazmat.2019.121271

Gul I, Le W, Jie Z et al (2021) Recent advances on engineered enzyme-conjugated biosensing modalities and devices for halogenated compounds. TrAC Trends Anal Chem 134:116145. https://doi.org/10.1016/j.trac.2020.116145

Karunatillaka I, Jaroszewski L, Godzik A (2020) Novel polyethylene terephthalate (PET) plastic degrading enzymes from the environmental metagenome. Authorea Preprints, Hoboken

Kawai F, Oda M, Tamashiro T et al (2014) A novel Ca2+-activated, thermostabilized polyesterase capable of hydrolyzing polyethylene terephthalate from Saccharomonospora viridis AHH190. Appl Microbiol Biotechnol 98:10053–10064

Kim JW, Park S-B, Tran Q-G et al (2020) Functional expression of polyethylene terephthalate-degrading enzyme (PETase) in green microalgae. Microb Cell Fact 19:1–9

Liu C, Shi C, Zhu S et al (2019) Structural and functional characterization of polyethylene terephthalate hydrolase from Ideonella sakaiensis. Biochem Biophys Res Commun 508:289–294

Ma Y, Yao M, Li B et al (2018) Enhanced poly (ethylene terephthalate) hydrolase activity by protein engineering. Engineering 4:888–893

Meng X, Yang L, Liu H et al (2021) Protein engineering of stable IsPETase for PET plastic degradation by Premuse. Int J Biol Macromol 180:667–676

Palm GJ, Reisky L, Böttcher D et al (2019) Structure of the plastic-degrading Ideonella sakaiensis MHETase bound to a substrate. Nat Commun 10:1–10

Prata JC, da Costa JP, Duarte AC et al (2019) Methods for sampling and detection of microplastics in water and sediment: a critical review. TrAC Trends Anal Chem 110:150–159. https://doi.org/10.1016/j.trac.2018.10.029

Ronkvist AM, Xie W, Lu W et al (2009) Cutinase-catalyzed hydrolysis of poly (ethylene terephthalate). Macromolecules 42:5128–5138

Shi L, Liu H, Gao S et al (2021) Enhanced extracellular production of IsPETase in Escherichia coli via engineering of the pElB signal peptide. J Agric Food Chem 69:2245–2252

Skoczinski P, Krause L, Raschka A et al (2021) Current status and future development of plastics: solutions for a circular economy and limitations of environmental degradation. Methods in enzymology, vol 648. Elsevier, Amsterdam, pp 1–26

Son HF, Cho IJ, Joo S et al (2019) Rational protein engineering of thermo-stable PETase from Ideonella sakaiensis for highly efficient PET degradation. ACS Catal 9:3519–3526

Tanamasuwarat S, Takehana T, Yoshida S et al (2016) Ideonella sakaiensis sp. nov., isolated from a microbial consortium that degrades poly (ethylene terephthalate). Int J Syst Evol Microbiol 66:2813–2818

Taniguchi I, Yoshida S, Hiraga K et al (2019) Biodegradation of PET: current status and application aspects. ACS Catal 9:4089–4105

Tokiwa Y, Calabia BP (2007) Biodegradability and biodegradation of polystyres. J Polym Environ 15:259–267
Wallace NE, Adams MC, Chafin AC et al (2020) The highly crystalline PET found in plastic water bottles does not support the growth of the PETase-producing bacterium *Ideonella sakaiensis*. Environ Microbiol Rep 12:578–582

Webb HK, Arnott J, Crawford RJ et al (2012) Plastic degradation and its environmental implications with special reference to poly(ethylene terephthalate). Polymers 5:1–18

Wei R, Zimmermann W (2017) Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate. Microb Biotechnol 10:1302

Widyastuti G (ed) (2018) Genetic engineered *Ideonella sakaiensis* bacteria: a solution of the legendary plastic waste problem. In proceedings of the 3rd international conference of integrated intellectual community, Hanover, Germany, 27–29 May 2018

Yang J, Yang Y, Wu W-M et al (2014) Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environ Sci Technol 48:13776–13784

Yoshida S, Hiraga K, Takehana T et al (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351:1196–1199

Yu Y, Lee C, Hwang S (2005) Analysis of community structures in anaerobic processes using a quantitative real-time PCR method. Water Sci Technol 52:85–91

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.