

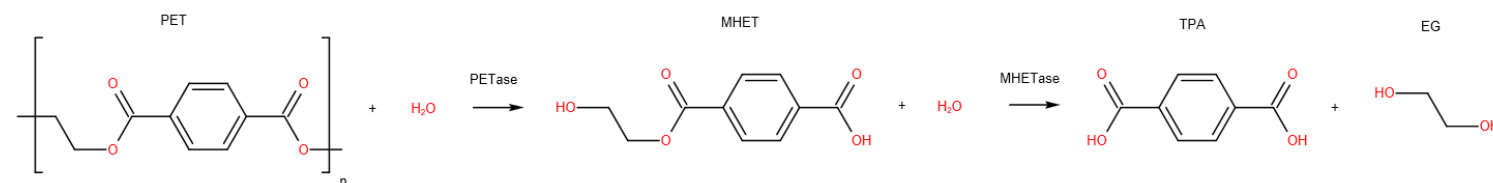
Quantifying PET Hydrolysis by *Ideonella sakaiensis* Using a Fluorescence Assay based on the Iron (II)-Catalyzed Radical Hydroxylation of Terephthalic Acid (TPA)

Introduction

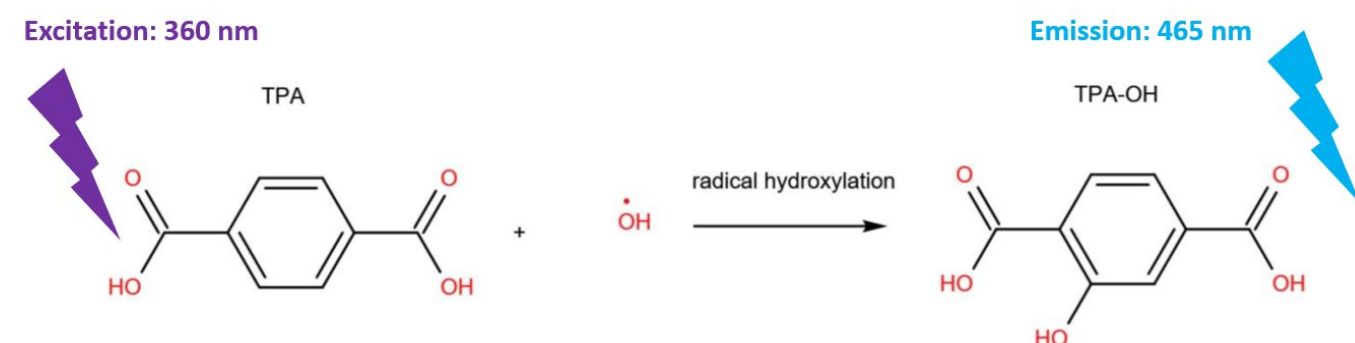
- In 2016, a PET-degrading microbe called *Ideonella sakaiensis* was discovered in Japan (Yoshida *et al.* 2016)



- I. sakaiensis* secretes **PETase** and **MHETase** that hydrolyze PET into Terephthalic Acid (TPA) and Ethylene Glycol (EG), so production of **TPA** is a signal of PET hydrolysis



- Current techniques used to quantify [TPA], such as GCMS and HPLC, are **cumbersome and slow**



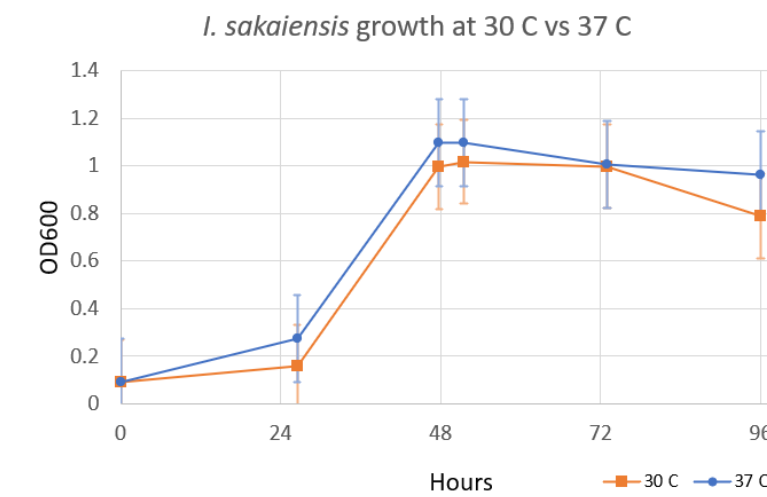
- Radical hydroxylation of TPA** produces a product with a strong and defined fluorescence signal at 465 nm (Barreto *et al.* 1994)

Conclusion

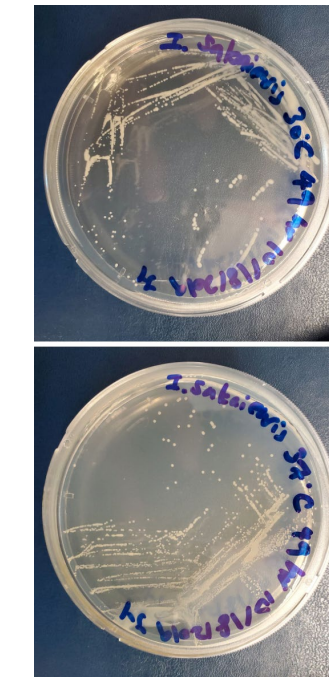
- TPA assay shows promising signs of detecting PET degradation over time, though visual signs of degradation was not observed
- Experiment should be repeated to investigate the high initial TPA signal anomaly
- Identity of *I. sakaiensis* in liquid culture should be confirmed using PCR
- OD600 should be tracked over time to test if *I. sakaiensis* used PET as a carbon source to grow

Culturing *Ideonella sakaiensis*

- Lyophilized *I. sakaiensis* was inoculated in NBRC 802 (growth) medium and incubated at 30 or 37 °C for 4 days to determine optimal growth conditions.

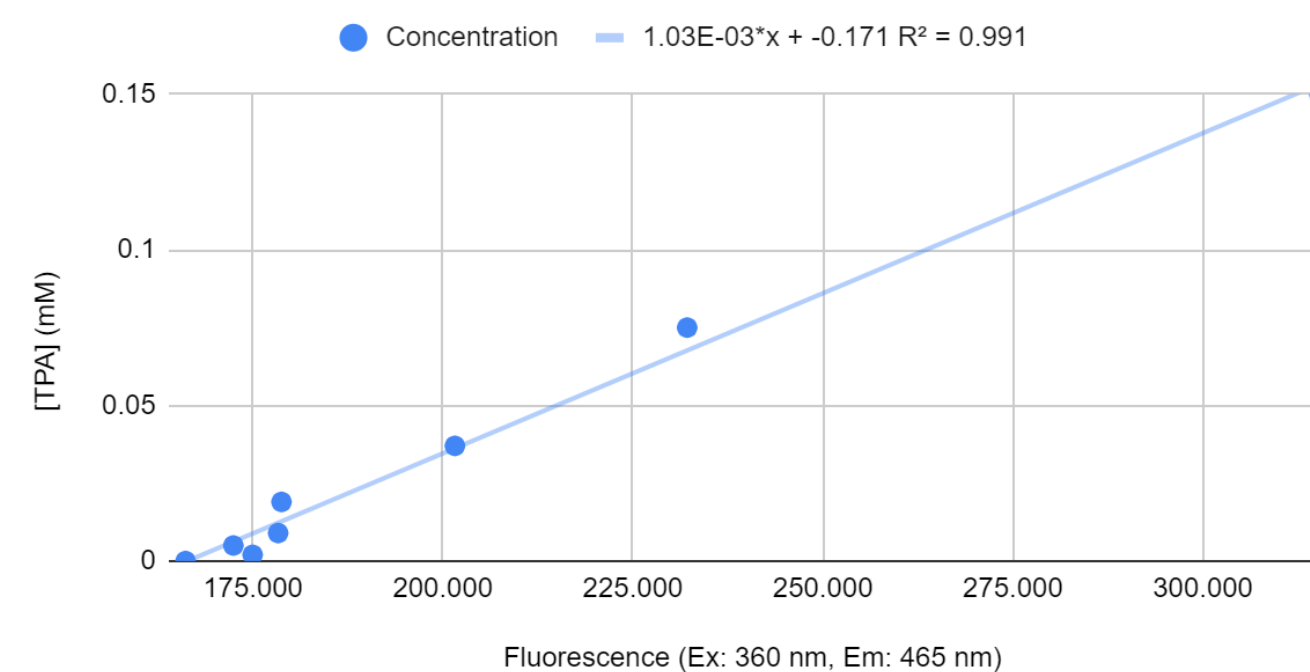


- I. sakaiensis* grows similarly at either 30 or 37 °C, with log phase occurring between 24-48 hours.
- I. sakaiensis* incubated at 30 (upper) or 37 °C (lower) formed colonies that appeared white, circular, and slightly raised.



TPA Fluorescence Assay

- TPA fluorescence standard curve was obtained by serially diluting TPA in YSV (minimal) medium and then reacting with FeSO₄ and EDTA for 15 minutes.

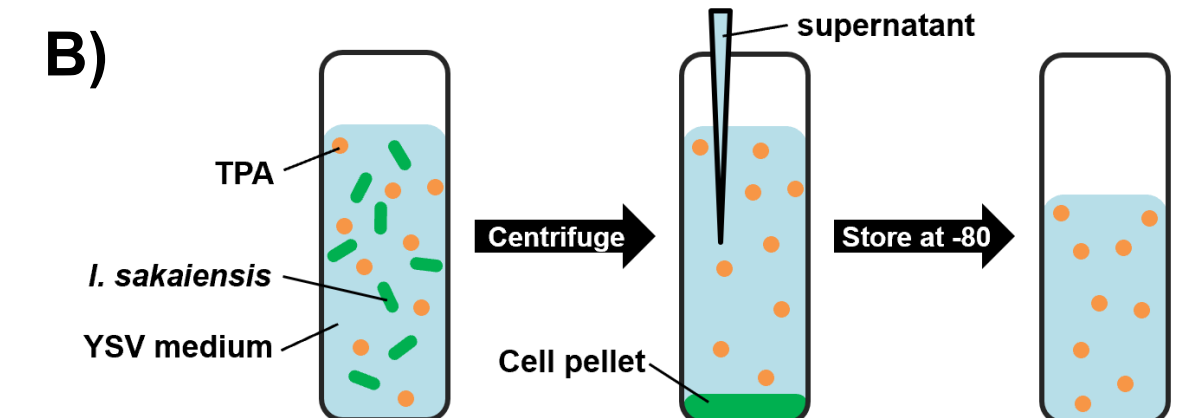
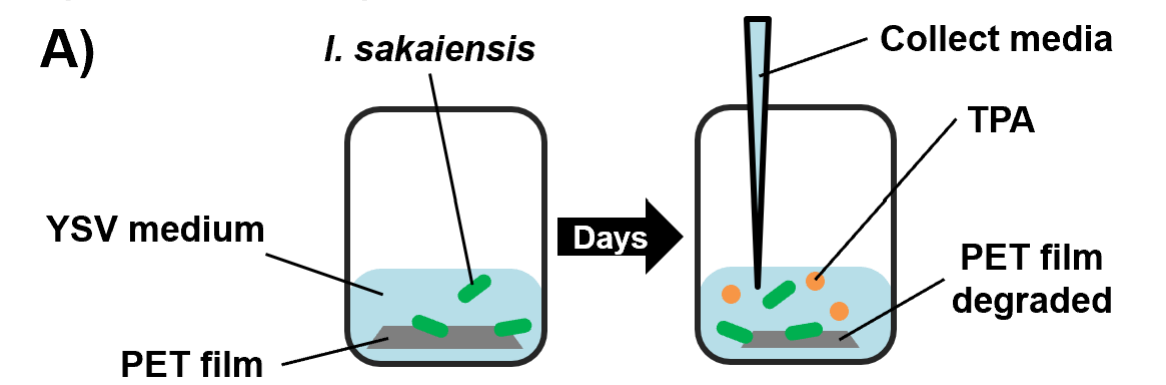


References

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- Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., ... & Oda, K. (2016). A bacterium that degrades and assimilates poly (ethylene terephthalate). *Science*, 351(6278), 1196-1199.

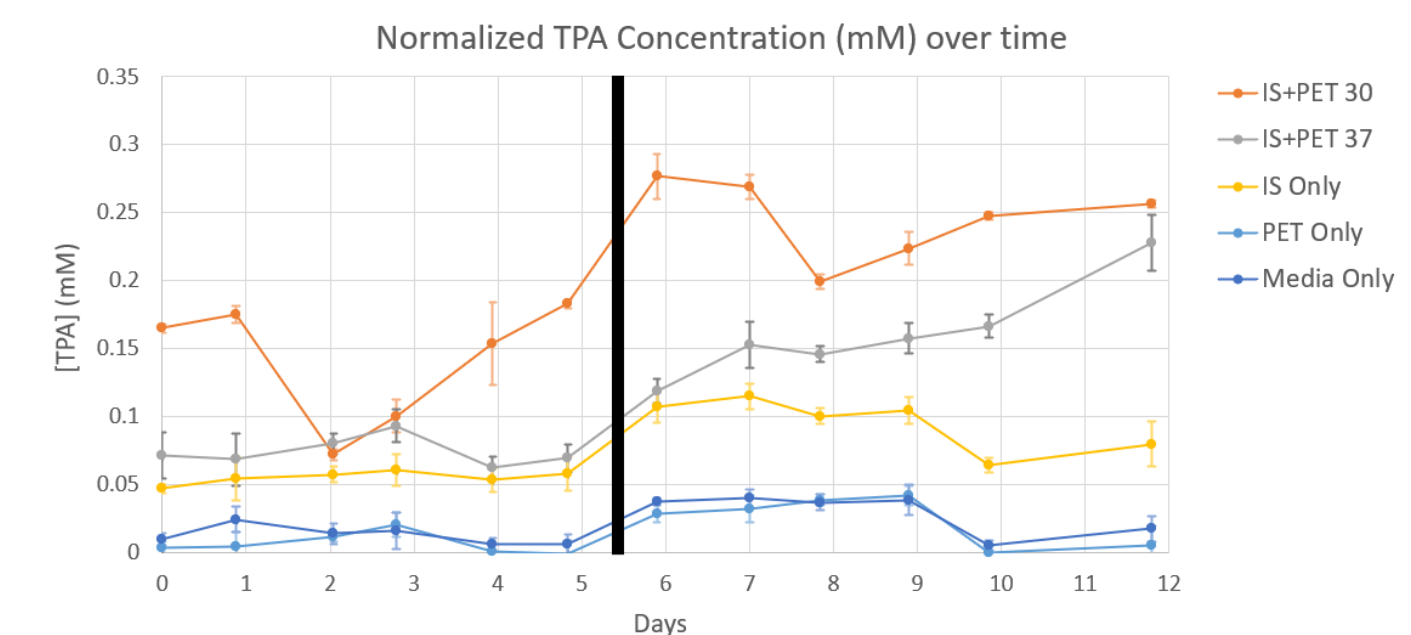
PET Hydrolysis by *Ideonella sakaiensis*

- I. sakaiensis* was first grown in NBRC 802 medium for 1-2 days then transferred to YSV (minimal) medium spiked with a piece of PET film.



- The media was collected once per day (A). Then, it was centrifuged and the supernatant containing TPA was stored at -80 °C until analysis (B).
- Five separate treatment groups were setup as follows.

Name	<i>Ideonella sakaiensis</i> ?	PET film?	Temp (°C)	Expected [TPA]
IS+PET 30	Yes	Yes	30	More TPA over time
IS+PET 37	Yes	Yes	37	Low TPA over time
IS only	Yes	No	30	No TPA
PET only	No	Yes	30	No TPA
Media only	No	No	30	No TPA



- The group where *Ideonella* was cultured with PET film at 30 °C showed increasing TPA over time, whereas control groups showed low background signal, suggesting PET was being hydrolyzed over time.