



Title: Genetic improvement of polyester degrading enzymes

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Editorial label ECORFAN: 607-8695
BCIERMMI Control Number: 2022-01
BCIERMMI Classification (2022): 261022-0001

Pages: 10
RNA: 03-2010-032610115700-14

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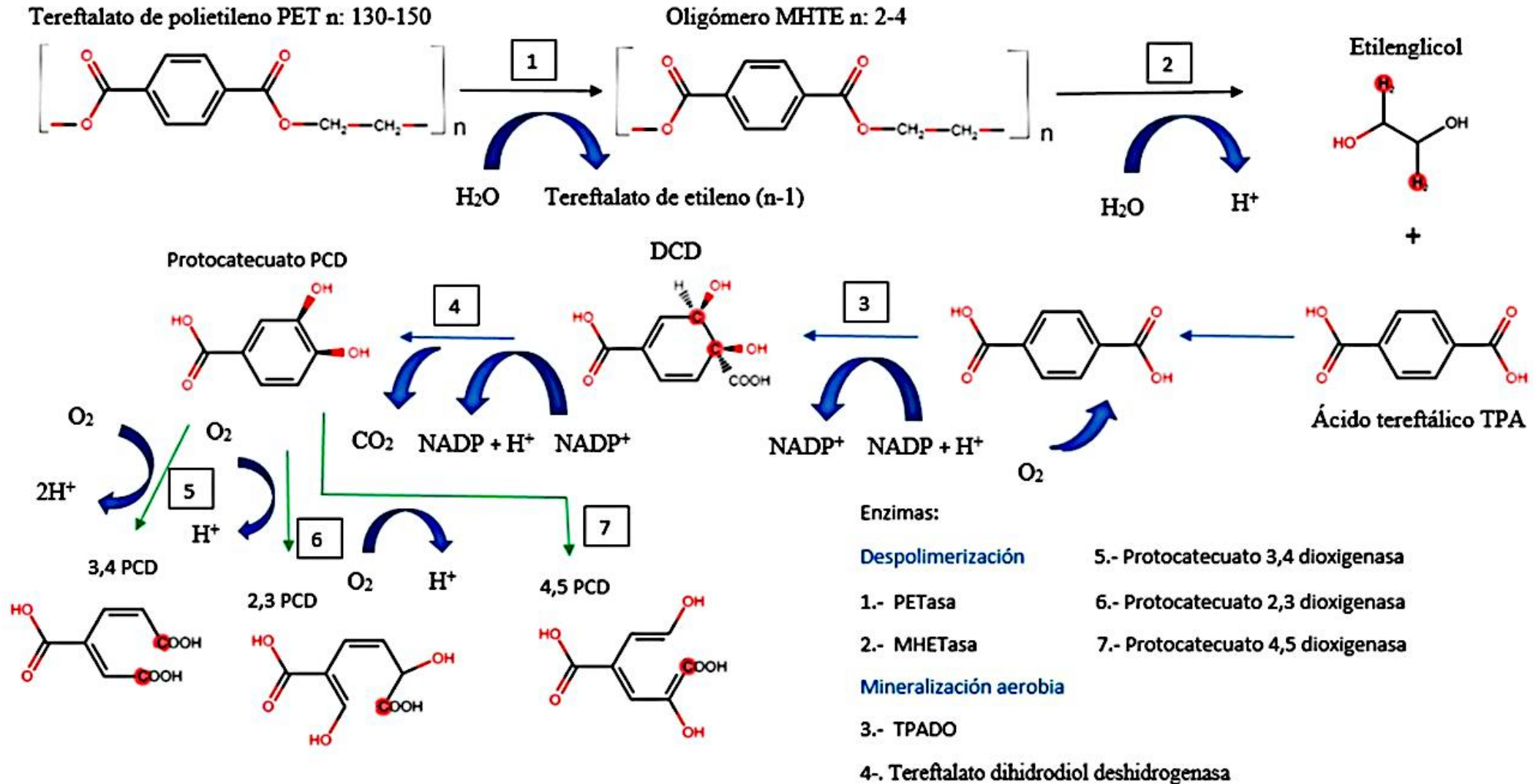
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Introduction



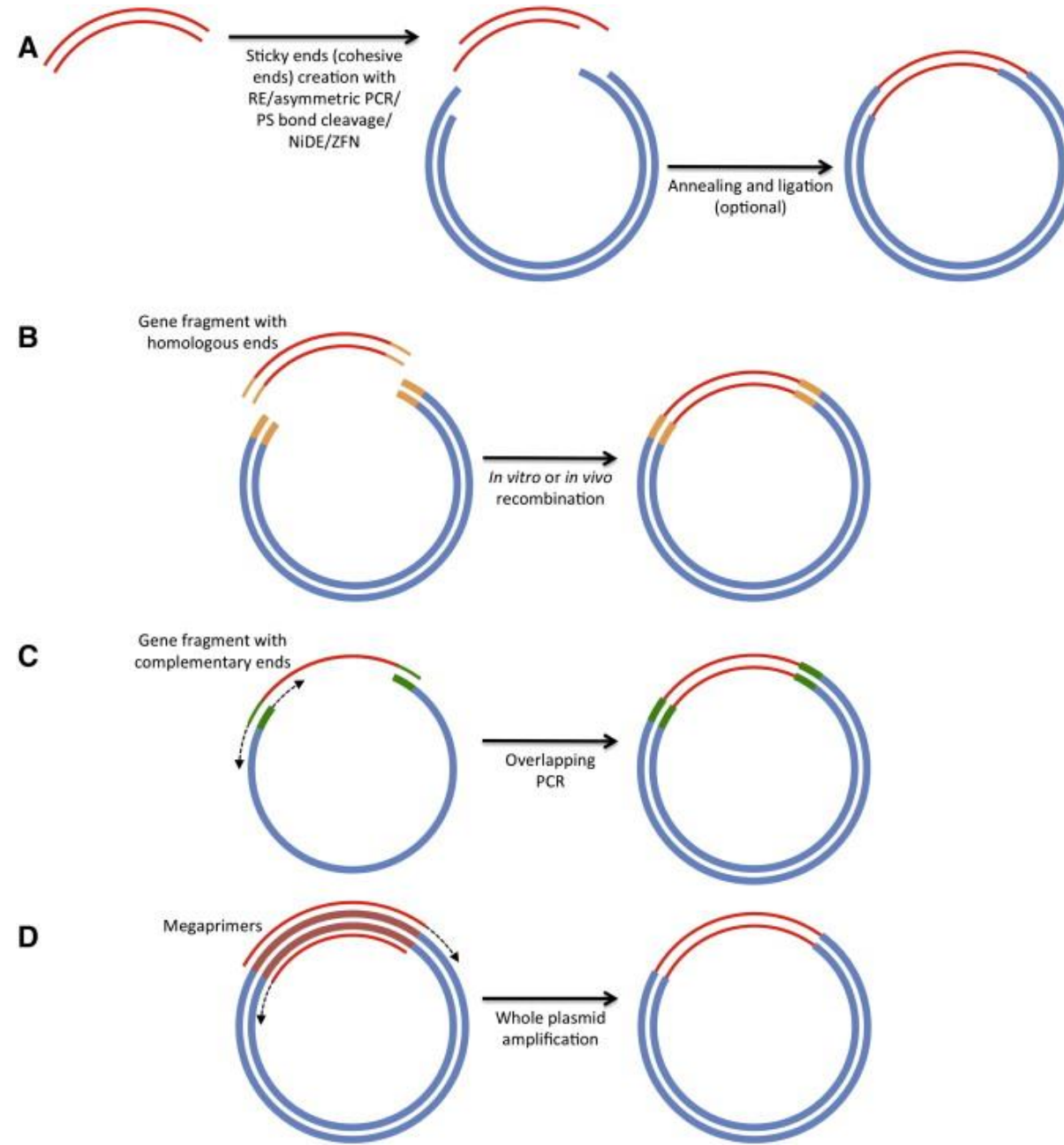
Procesos de degradación de poliésteres



Enzimas degradadoras de poliéster

Enzyme		pH	T	polyester
Lipase	Candida cylindracea	7	30	Poli(succinato de butilenoco-adipato de butileno)
Esteresas	Mucor miehei	7	37	Poli(ácido láticoco-glicólico)
cutinasa	CUTMR	8	30	PET,PCL y PES

Site-directed mutagenesis

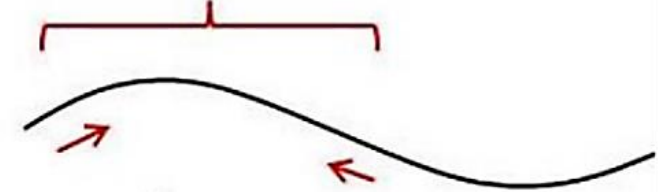


Error-prone PCR

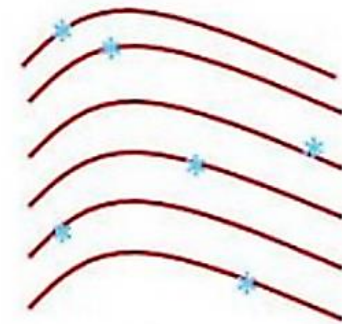
This method is used to introduce random mutations in a DNA segment >100 bp that are too long to be synthesized chemically (Wilson and Keefe et al., 2000).

error-prone PCR

target region for random mutagenesis



DNA polymerase;
high Mg^{2+}
 Mn^{2+}



transform into bacteria to
generate mutant library

Modified enzymes for the degradation of polyesters.

Enzyme	Mutation	Substrate	REF
IsPETase	C203S, C239S, W185A, S214H, I208A, W159A, M161A, Y87A, T88A, W168H.	PET Film	Han <i>et al.</i> , 2017
IsPETase	S160A, D206A, H237A, Y87A, M161A, W185A, I208A, W159A, S238A, N241A, R280A, C203S, C239S, S238F, W159H	PET Film	Joo <i>et al.</i> , 2018
IsPETase	S238F/W159H, W185A.	PET Film	Austin <i>et al.</i> , 2018
IsPETase	P181A, S121D/D186H, S121E, D186H, D186F, P181G, P181S, P181A/S121D/D186H.	PET Film	Son <i>et al.</i> , 2019
IsPETase	S160A, D206A, H237A, Y87B, M161A, W185A, I208A, W159A, S238A, N241A, R280A, C203S, C239S, S238F, W159H.	BHET	Sagong <i>et al.</i> , 2020
IsPETase	S160A, D206A, H237A, W159A, W159H, M161A, A209I, Q119A.	BHET	Liu <i>et al.</i> , 2018
IsMHETase	R411K, F415S, F424D, F424E, F424H, F424I, F424L, F424N, F424T, F424V, R411K/F424N, R411K/F424V, F415H/F424N.	MHET	Sagong <i>et al.</i> , 2020

Conclusions

The enzymes have been improved to meet industrial requirements. These improvements have been made through various molecular techniques, such as site-directed mutagenesis and error-prone PCR. For the modifications it is necessary to consider strategies and selective practices imitating the evolutionary processes of nature, thus creating enzymes with new characteristics of industrial interest.

References

Moharir, R. V., and Kumar, S. (2019). Challenges associated with plastic waste disposal and allied microbial routes for its effective degradation: a comprehensive review. *J. Clean. Prod.* 208, 65–76. DOI:

<https://doi.org/10.1016/j.jclepro.2018.10.059>; URL:

<https://www.sciencedirect.com/science/article/abs/pii/S0959652618330737>

Tee, K. L., & Wong, T. S. (2013). Polishing the craft of genetic diversity creation in directed evolution. *Biotechnology advances*, 31(8), 1707-1721. DOI: 10.1016/j.biotechadv.2013.08.021 URL:

<https://pubmed.ncbi.nlm.nih.gov/24012599/>.

Wilson, D. S., & Keefe, A. D. (2000). Random mutagenesis by PCR. *Current protocols in molecular biology*, 51(1), 8-3. DOI:

<https://doi.org/10.1002/0471142727.mb0803s51>;

Nikolic, Marija S., and Jasna Djonlagic. "Synthesis and characterization of biodegradable poly (butylene succinate-co-butylene adipate) s." *Polymer Degradation and Stability* 74.2 (2001): 263-270. [https://doi.org/10.1016/S0141-](https://doi.org/10.1016/S0141-3910(01)00156-2)

[3910\(01\)00156-2](https://doi.org/10.1016/S0141-3910(01)00156-2)

Kemme, M., Prokesch, I., & Heinzl-Wieland, R. (2011). Comparative study on the enzymatic degradation of poly (lactic-co-glycolic acid) by hydrolytic enzymes based on the colorimetric quantification of glycolic acid. *Polymer testing*, 30(7), 743-748. <https://doi.org/10.1016/j.polymertesting.2011.06.009>

REFERENCE

- Eberl, A., Heumann, S., Kotek, R., Kaufmann, F., Mitsche, S., Cavaco-Paulo, A., & Gübitz, G. M. (2008). Enzymatic hydrolysis of PTT polymers and oligomers. *Journal of Biotechnology*, 135(1), 45-51. <https://doi.org/10.1016/j.jbiotec.2008.02.015>
- Han, X.; Liu, W.; Huang, J.-W.; Ma, J.; Zheng, Y.; Ko, T.-P.; Xu, L.; Cheng, Y.-S.; Chen, C.-C.; Guo, R.-T. Structural Insight into Catalytic Mechanism of PET Hydrolase. *Nat. Commun.*, 2017, 8, 2106. DOI: [10.1038/s41467-017-02255-z](https://doi.org/10.1038/s41467-017-02255-z) URL: <https://pubmed.ncbi.nlm.nih.gov/29235460/>
- Joo, S.; Cho, I.J.; Seo, H.; Son, H.F.; Sagong, H.-Y.; Shin, T.J.; Choi, S.Y.; Lee, S.Y.; Kim, K.-J. Structural Insight into Molecular Mechanism of Poly(Ethylene Terephthalate) Degradation. *Nat. Commun.*, 2018, 9, 382. DOI: <https://doi.org/10.1038/s41467-018-02881-1> URL: <https://www.nature.com/articles/s41467-018-02881-1>
- Austin, H.P.; Allen, M.D.; Donohoe, B.S.; Rorrer, N.A.; Kearns, F.L.; Silveira, R.L.; Pollard, B.C.; Dominick, G.; Duman, R.; El Omari, K.; Mykhaylyk, V.; Wagner, A.; Michener, W.E.; Amore, A.; Skaf, M.S.; Crowley, M.F.; Thorne, A.W.; Johnson, C.W.; Woodcock, H.L.; McGeehan, J.E.; Beckham, G.T. Characterization and Engineering of a Plastic-Degrading Aromatic Polyesterase. *Proc. Natl. Acad. Sci. U. S. A.*, 2018, 115, E4350–E4357. <https://doi.org/10.1073/pnas.1718804115>; URL: <https://www.pnas.org/doi/10.1073/pnas.1718804115>

REFERENCE

Son, H.F.; Cho, I.J.; Joo, S.; Seo, H.; Sagong, H.-Y.; Choi, S.Y.; Lee, S.Y.; Kim, K.-J. Rational Protein Engineering of Thermo-Stable PETase from Ideonella Sakaiensis for Highly Efficient PET Degradation. ACS Catal., 2019, 9, 3519–3526. DOI: <https://doi.org/10.1021/acscatal.9b00568>
URL: <https://pubs.acs.org/doi/abs/10.1021/acscatal.9b00568>.



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