Jennifer Chow, Pablo Pérez-García, Robert Dierkes, Hongli Zhang, Wolfgang R. Streit

Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

FuturEnzyme
Technologies of the FUTURe for low-cost ENZYMEs for environment-friendly products

Mining the microbial diversity for esterases, lipases and plastic degrading enzymes

CLIB Event

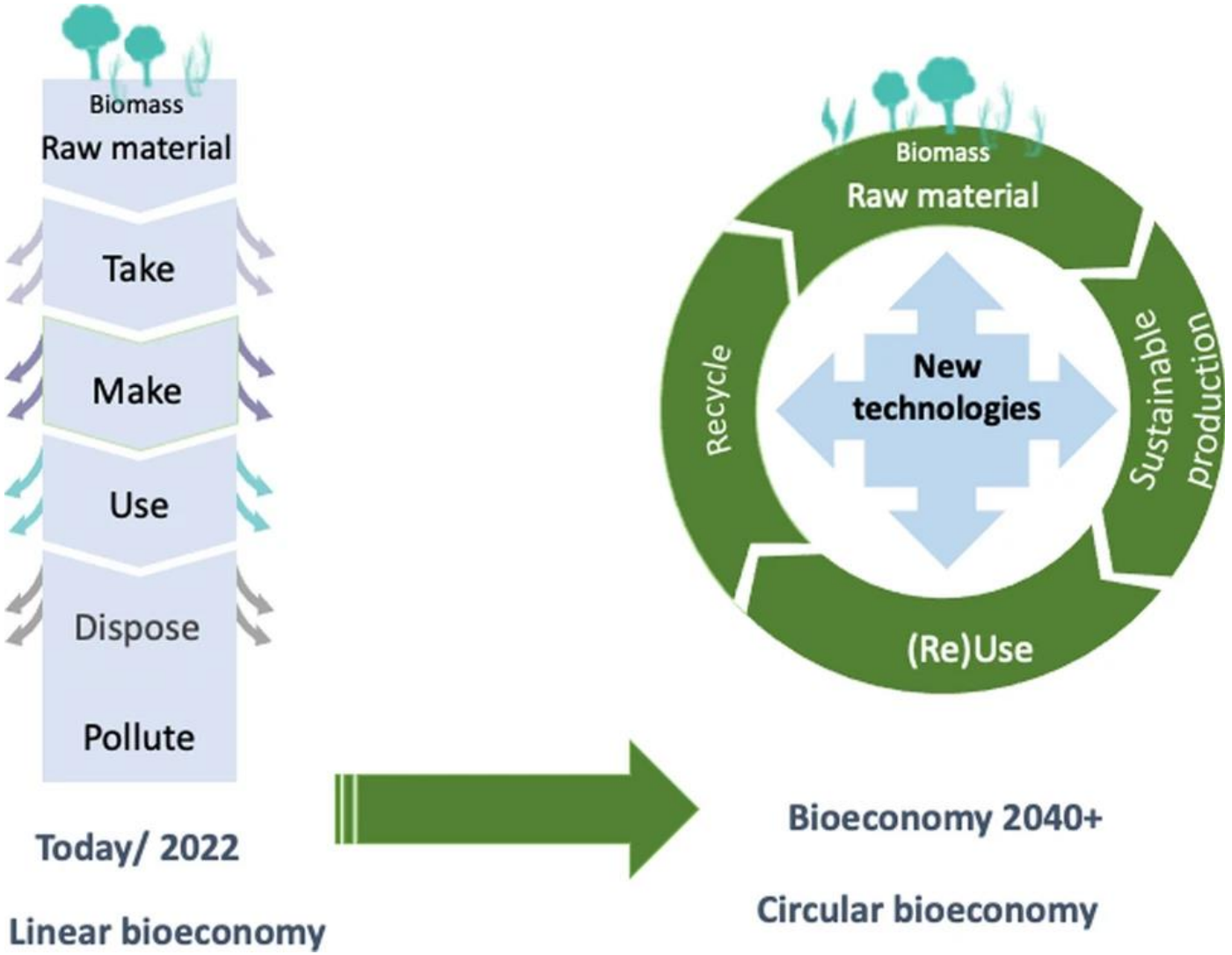
Fantastic enzymes:

Where and how to find them

Project funded by the European Union's Horizon 2020
Research and Innovation Programme under grant agreement No [101000327]

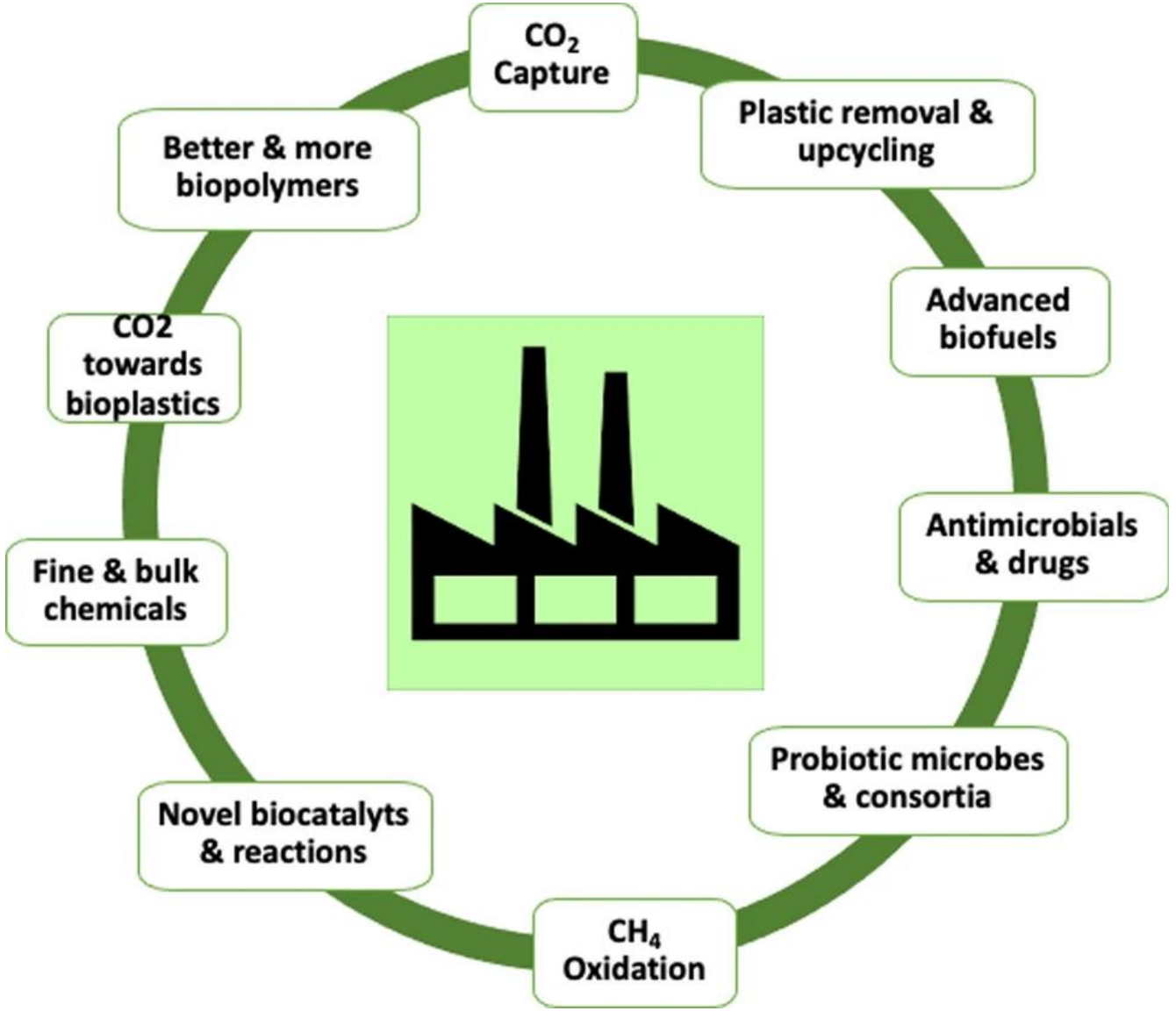
6th of September 2022

Shifting towards a circular bioeconomy



Antranikian, G., Streit, W.R. *Extremophiles* **26**, 10 (2022). https://doi.org/10.1007/s00792-022-01261-4

Key developments for a sustainable economy



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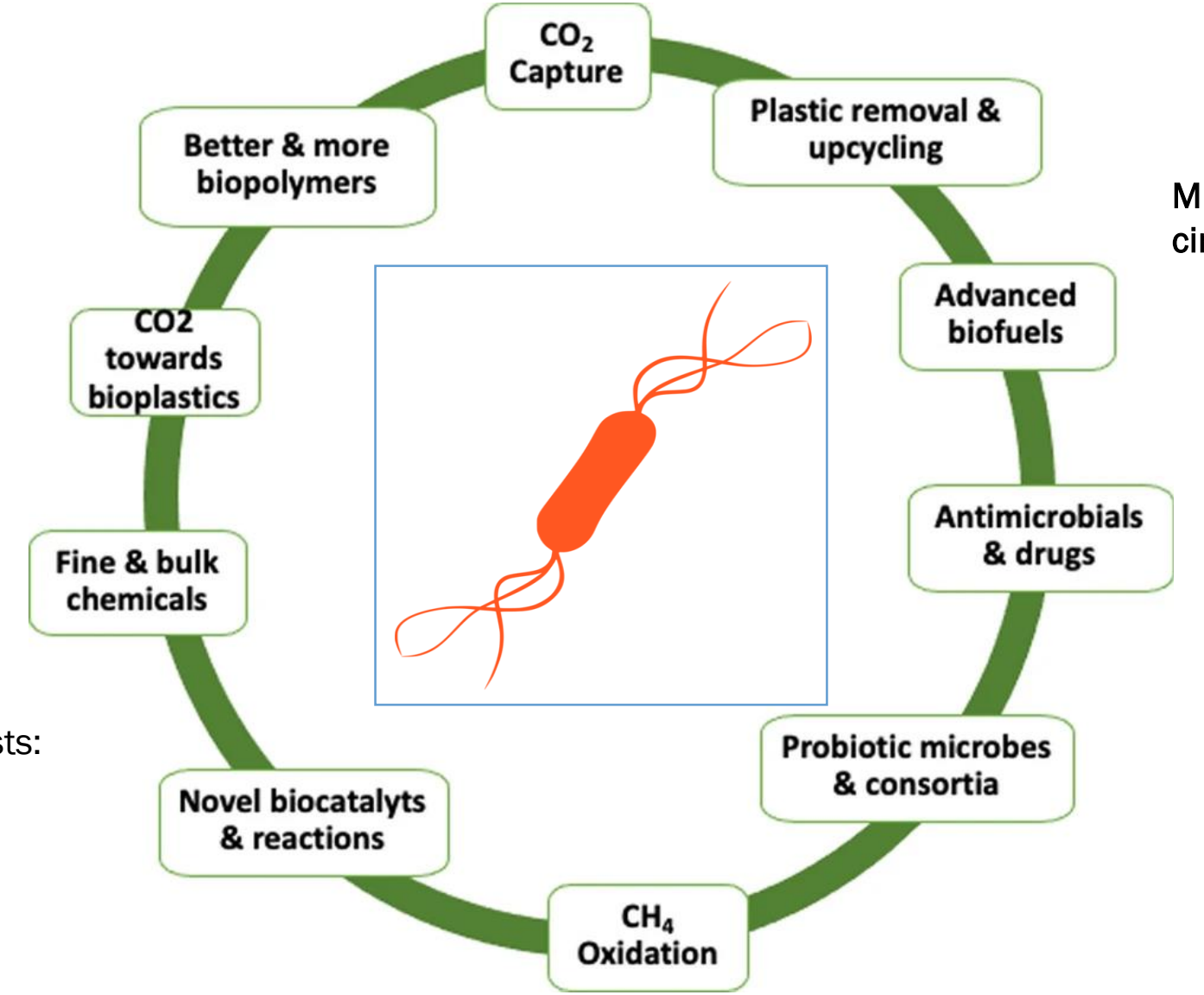
Key developments for a sustainable economy

Global biocatalysts market 2020: **117 kilotons**

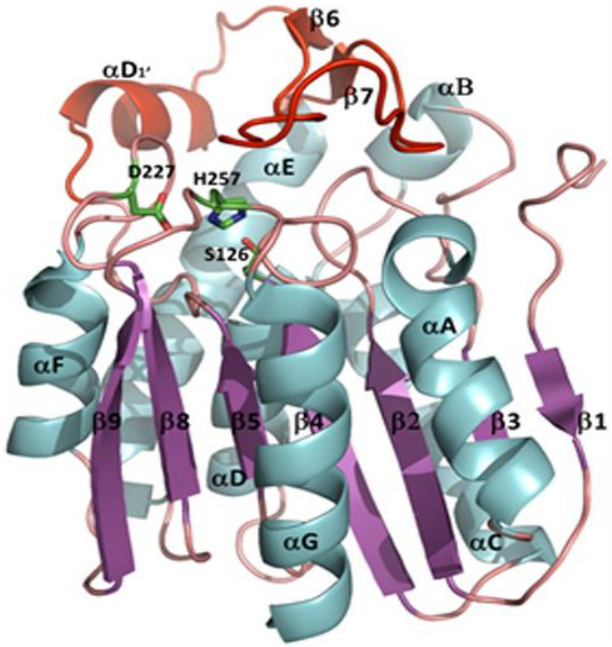
Dominant type of biocatalysts: **Hydrolases**
https://www.expertmarketresearch.com/



Microbes harbour the key to a circular economy

Antranikian, G., Streit, W.R. *Extremophiles* **26**, 10 (2022). https://doi.org/10.1007/s00792-022-01261-4

Lipases and carboxylesterases in biotechnology



Food
Pasta and bread dough, oils and fats, drinks

Washing detergents

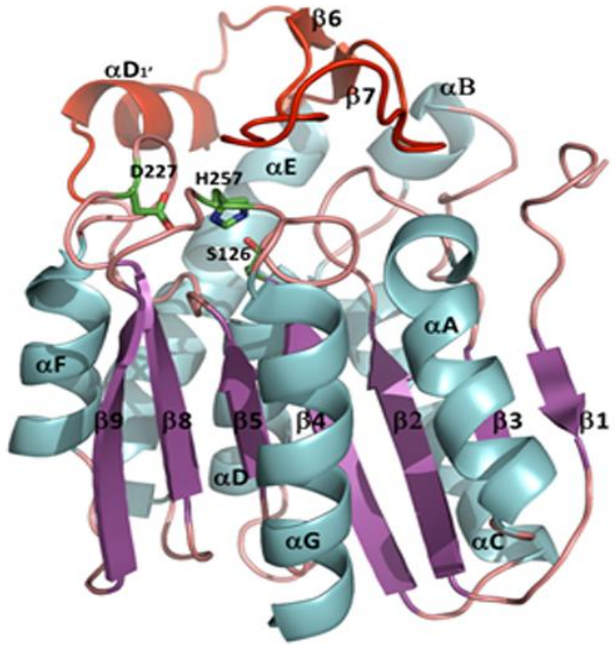
Biodiesel

Textile-, paper production

Organic Syntheses
Polyesters,
Pharmaceuticals, basic chemicals

Cosmetics

Lipases and carboxylesterases in microbes



Versatile biocatalysts

- Highly specific or promiscuous
- Secreted enzymes can be highly stable

Metabolism
Lipid degradation
Lipid modification
Membrane adaptation…

Growth promotion
Carbon source
Degradation of toxins
Quorum Quenching
Colonization of niches
Symbiosis…

Virulence
Degradation of the plant cell wall
Production of surface-active substances
Degradation of host lipids…

Metagenomics

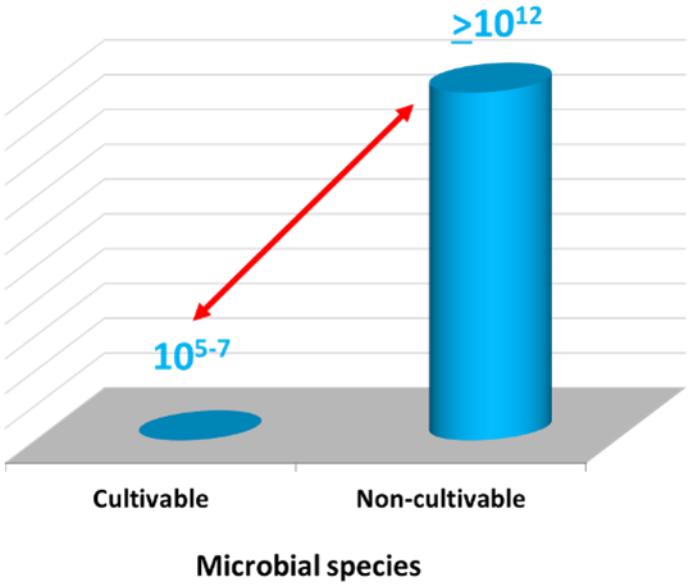
Bacteria/Archaea: 10^{30} cells on earth

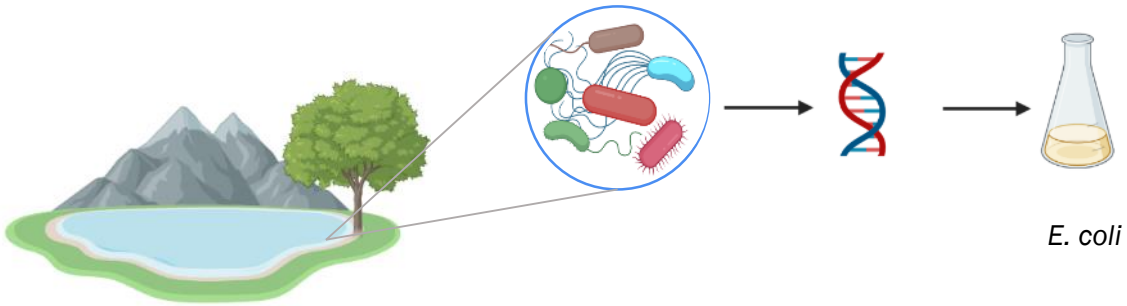
10^{7-12} microbial species

10^{10-15} bacterial/archaeal enzymes

Billions of different reactions

Billions of potential biomolecules/compounds for different industrial applications



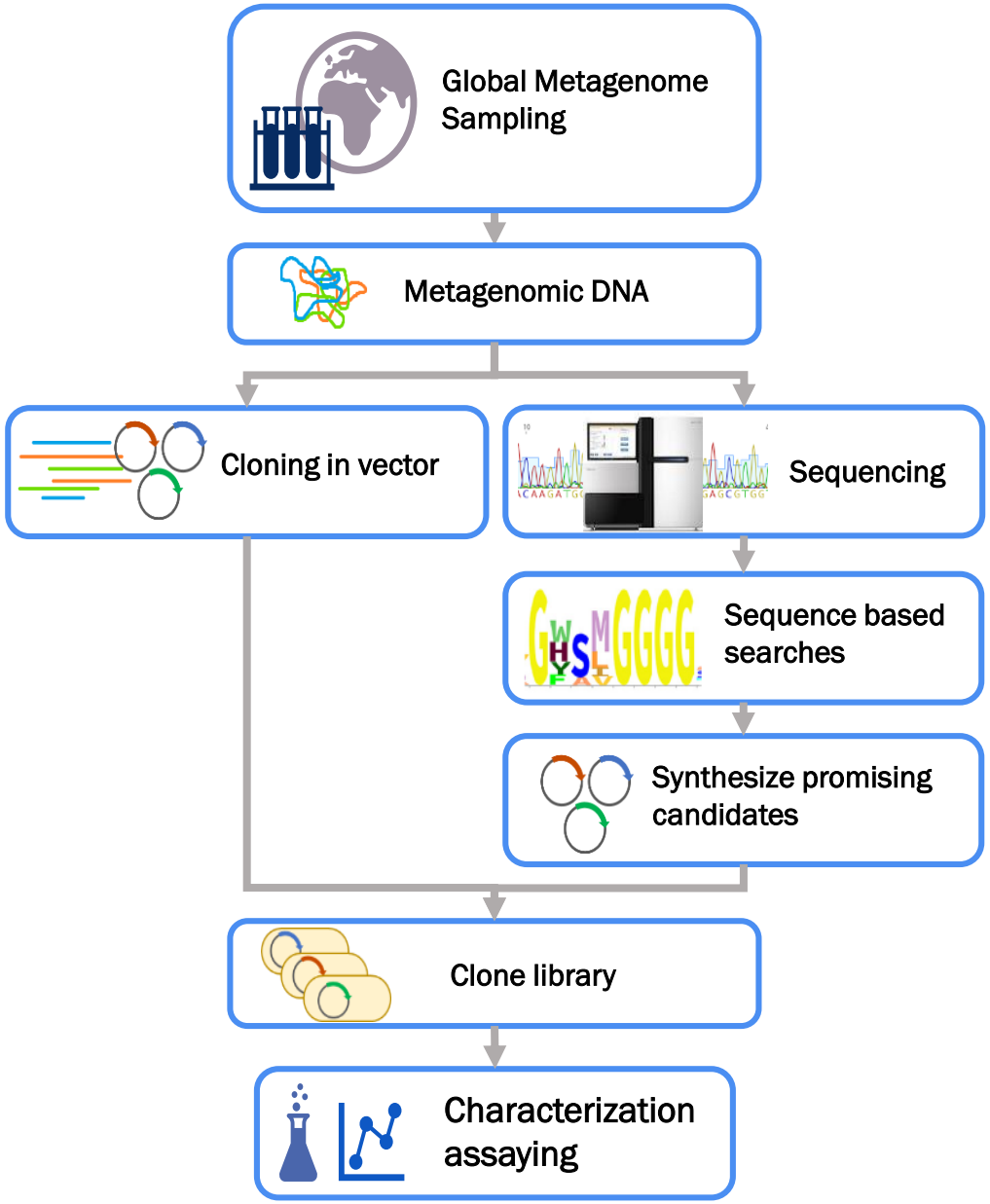


E. coli

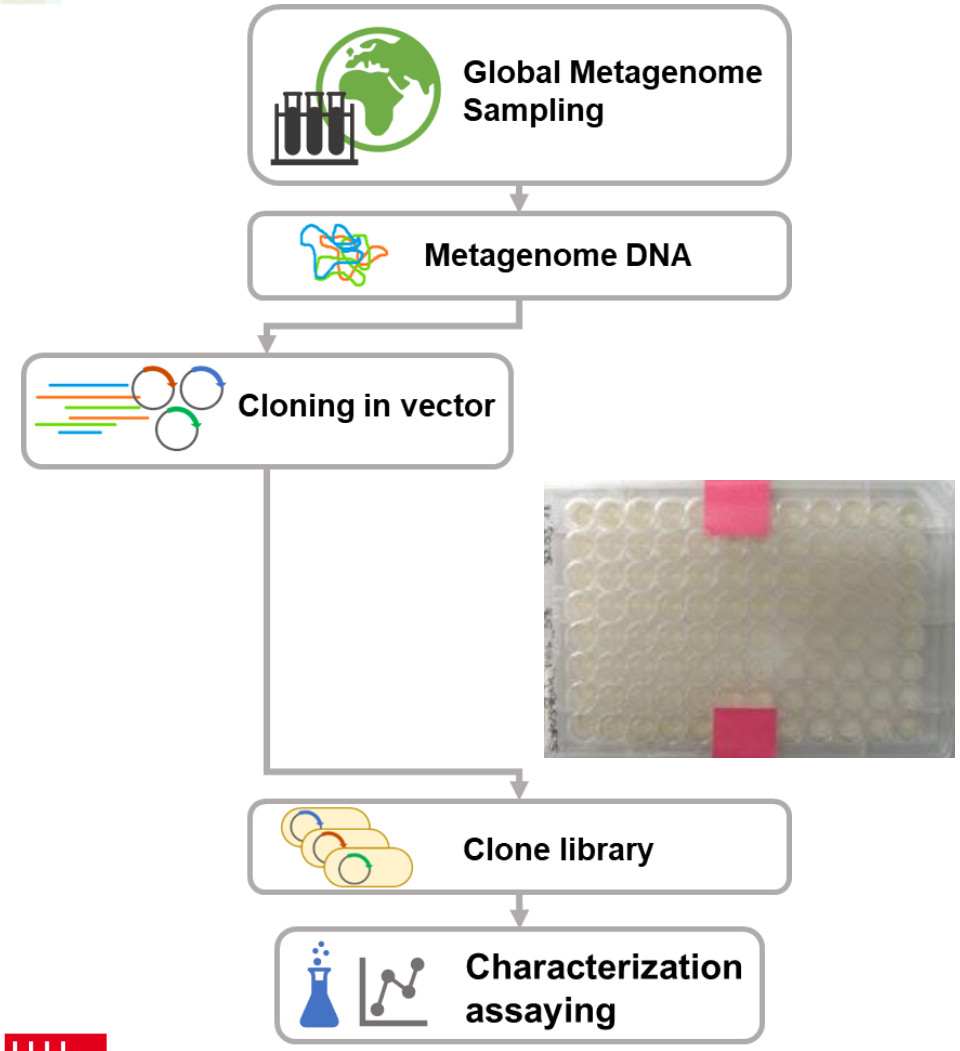
Metagenome: The entirety of mixed microbial communities genomes

Direct DNA extraction from environmental samples without prior cultivation

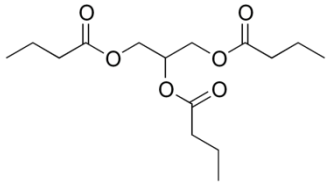
Metagenomics



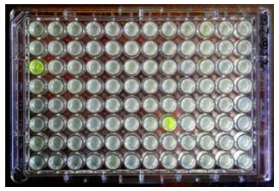
Lipase toolbox @UHAM

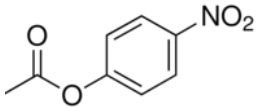


Tributyrin (TBT)



para-Nitrophenol (*p*NP)





Metagenome library	No. of clones
Elephant faeces	20,064
Teufelsbrück, river Elbe sediment	20,256
Glückstadt, river Elbe sediment	23,520
Algae photobioreactor biofilm	14,976
Esterase-lipase-box (collection of metagenome-derived enzymes)	81
Total = 78,897	

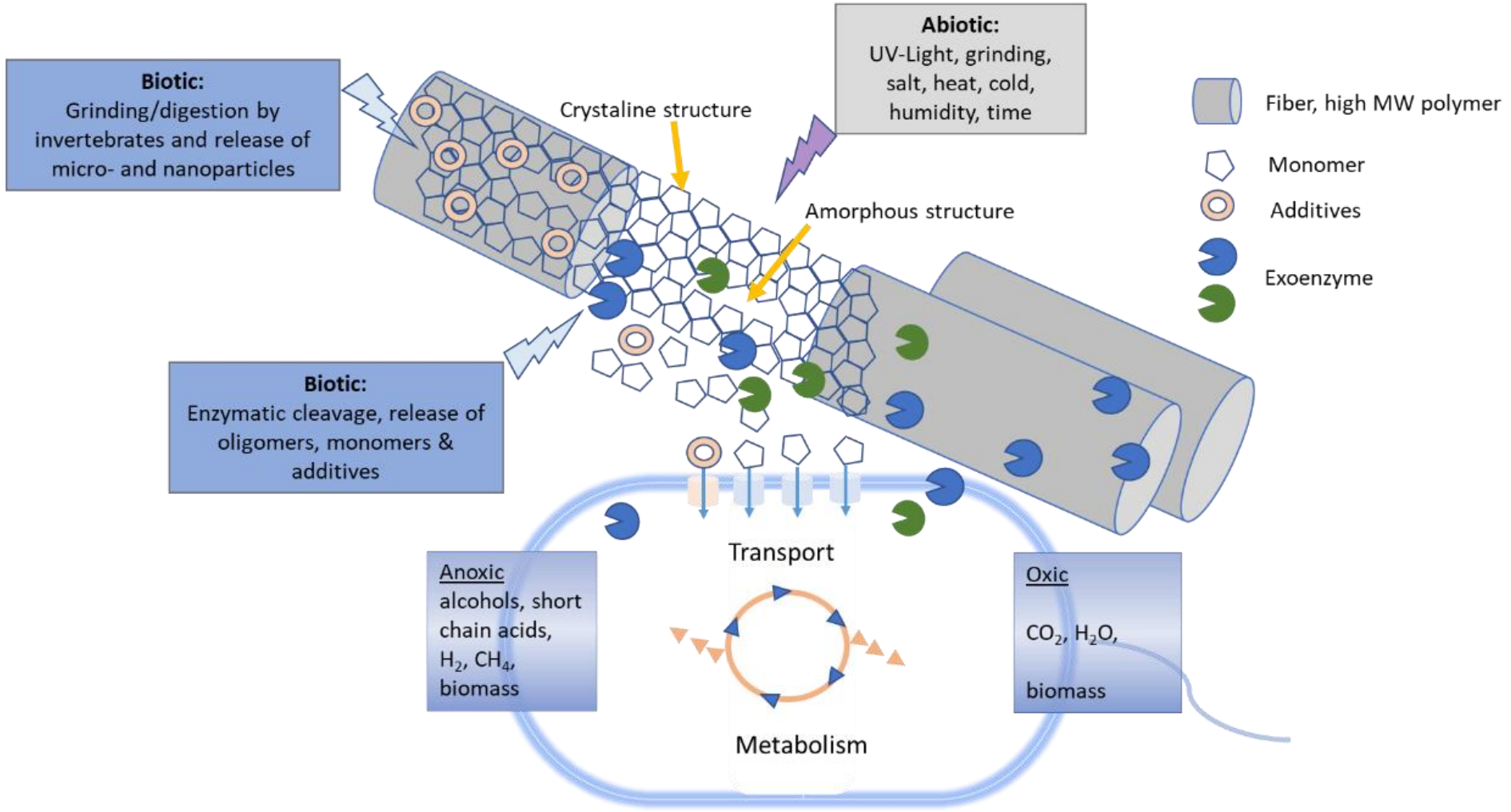
Plastics: A global challenge & no effective solutions

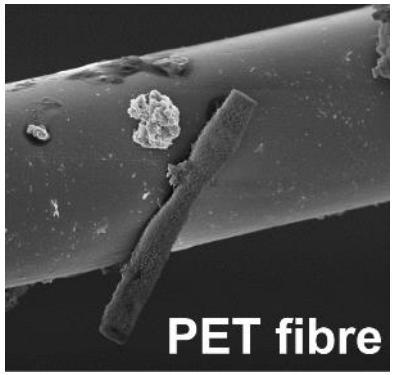
- 380-450 Mio tons produced annually
- 50% single use products
- Recycling rate less than 10 %
- Much of it ends up in terrestrial & aquatic environments
- Microplastics most likely pose the far more serious problem
- We eat more than 20 kg of plastic in our life

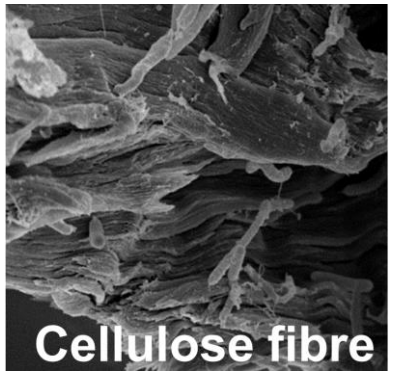
Microbial enzymes as promising approach to plastic waste reduction

MacArthur Foundation, Plasticseurope, Plasticsocean, National Geographics, Renkgroup.com

A model for microbial plastic degradation

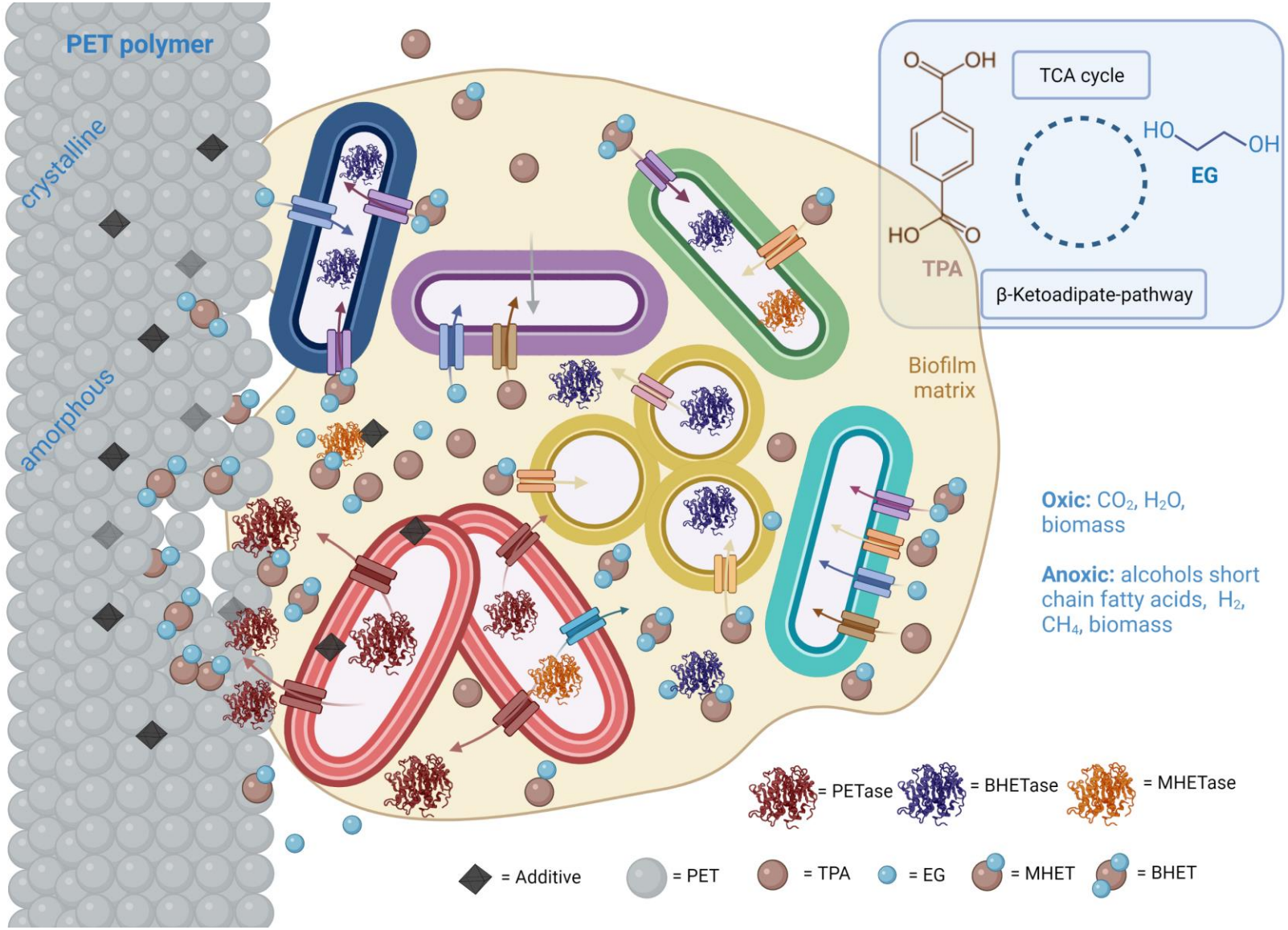






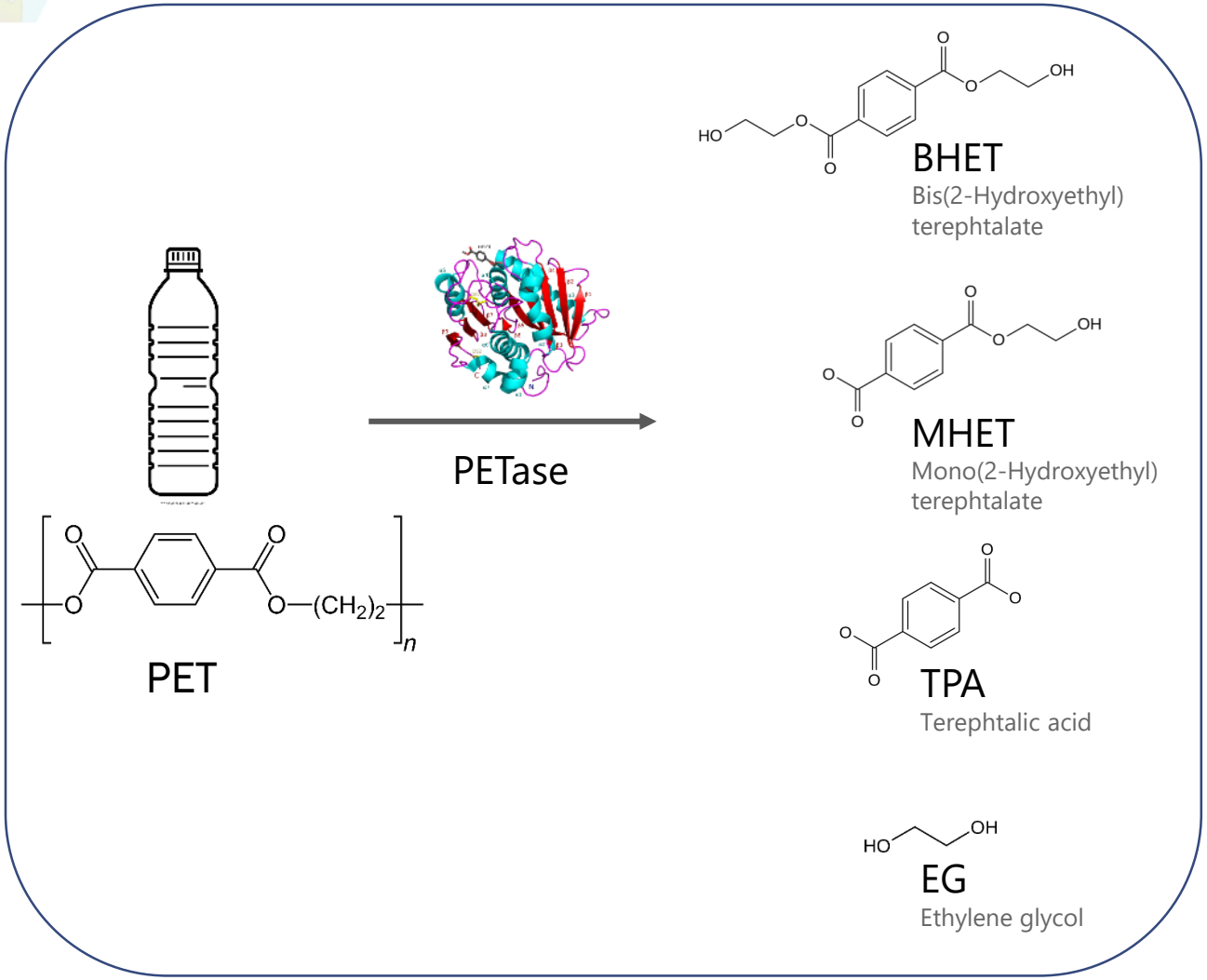
Zhang et al., 2021 ASM Handbook, in revision; Danso et al., 2019 AEM

Microbial degradation – a community job?



Chow, J., Perez-Garcia, P., Dierkes R. and Streit W.R. (2022) *Microbial Biotechnology* in press

PET hydrolases



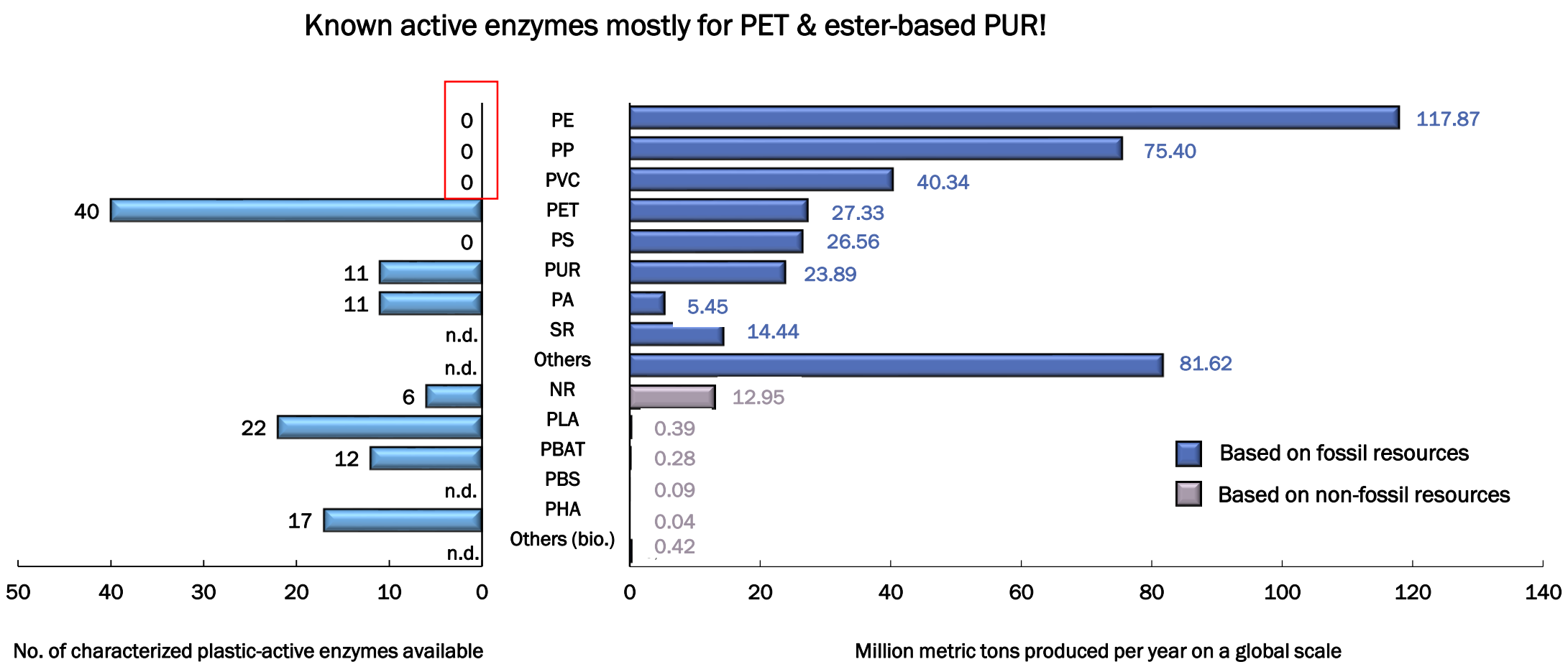
Best performing enzymes on PET

- **LCC** - Leaf compost cutinase - Actinobacteria (Sulaiman, 2012)
- ***Is*PETase** – *Ideonella sakaiensis* -Proteobacteria (Yoshida 2016)

Application in bioindustries

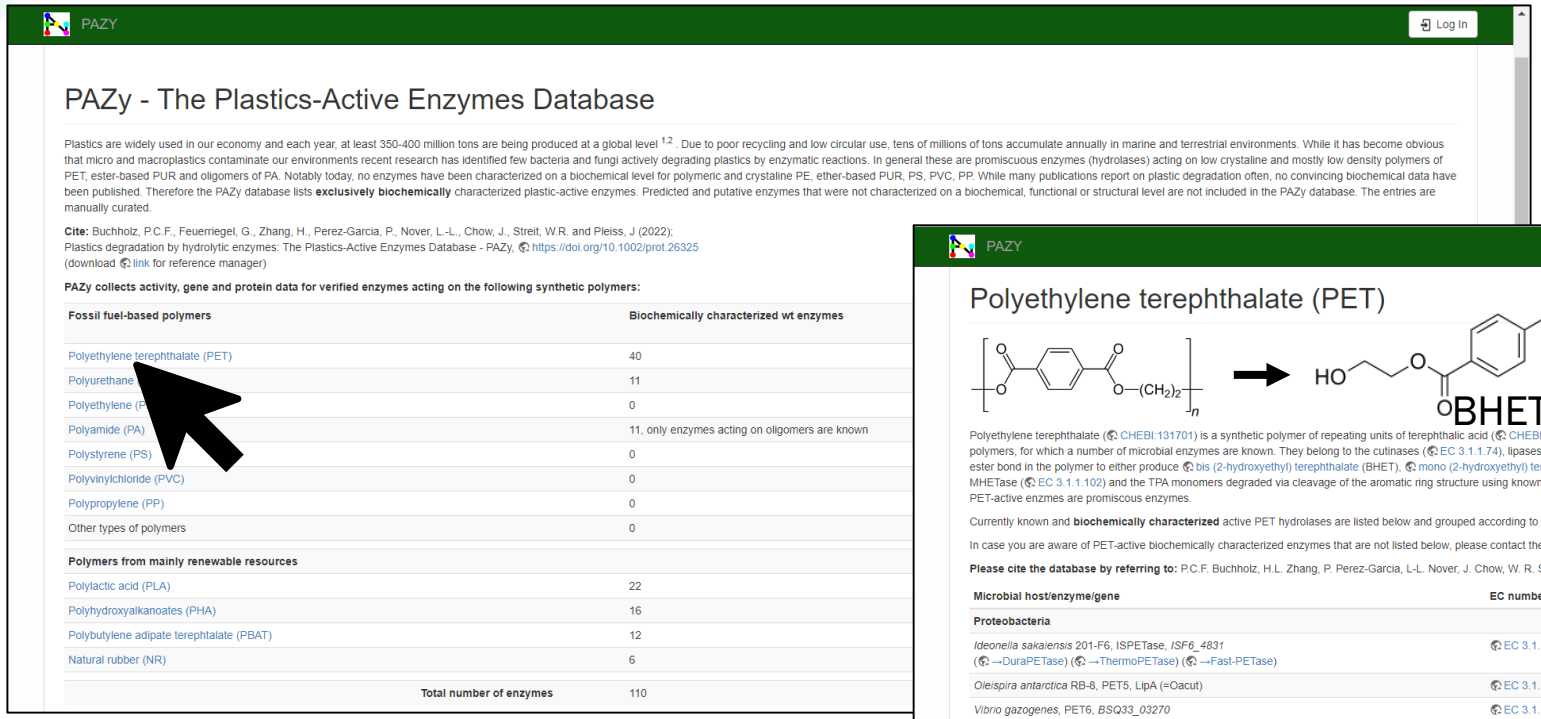
- Reduction of waste streams
- Re-valorization
- Reduction of microplastics in waste water

Enzymatic plastic depolymerization – Where are we?

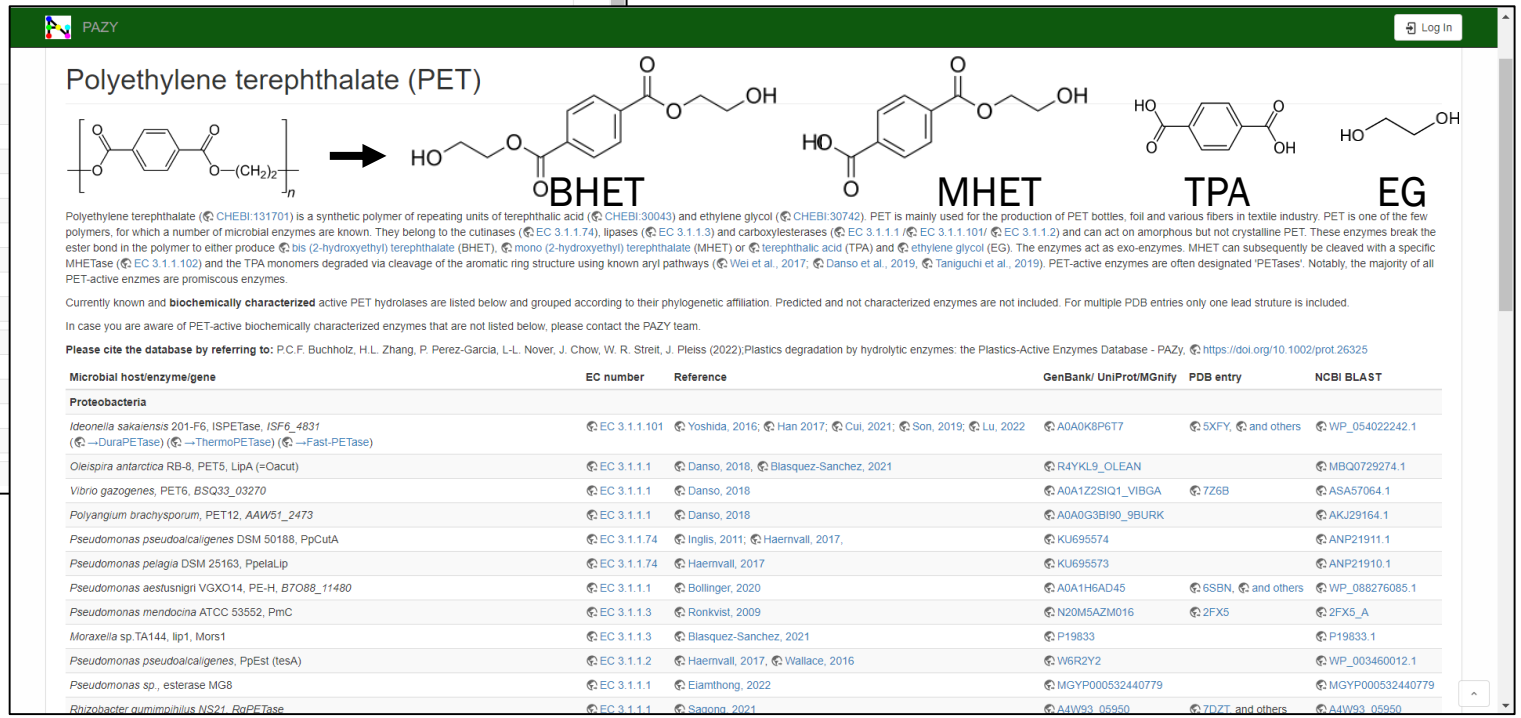


Chow, J., Perez-Garcia, P., Dierkes R. and Streit W.R. (2022) *Microbial Biotechnology* in press

PAZy - The Plastics-Active Enzymes Database



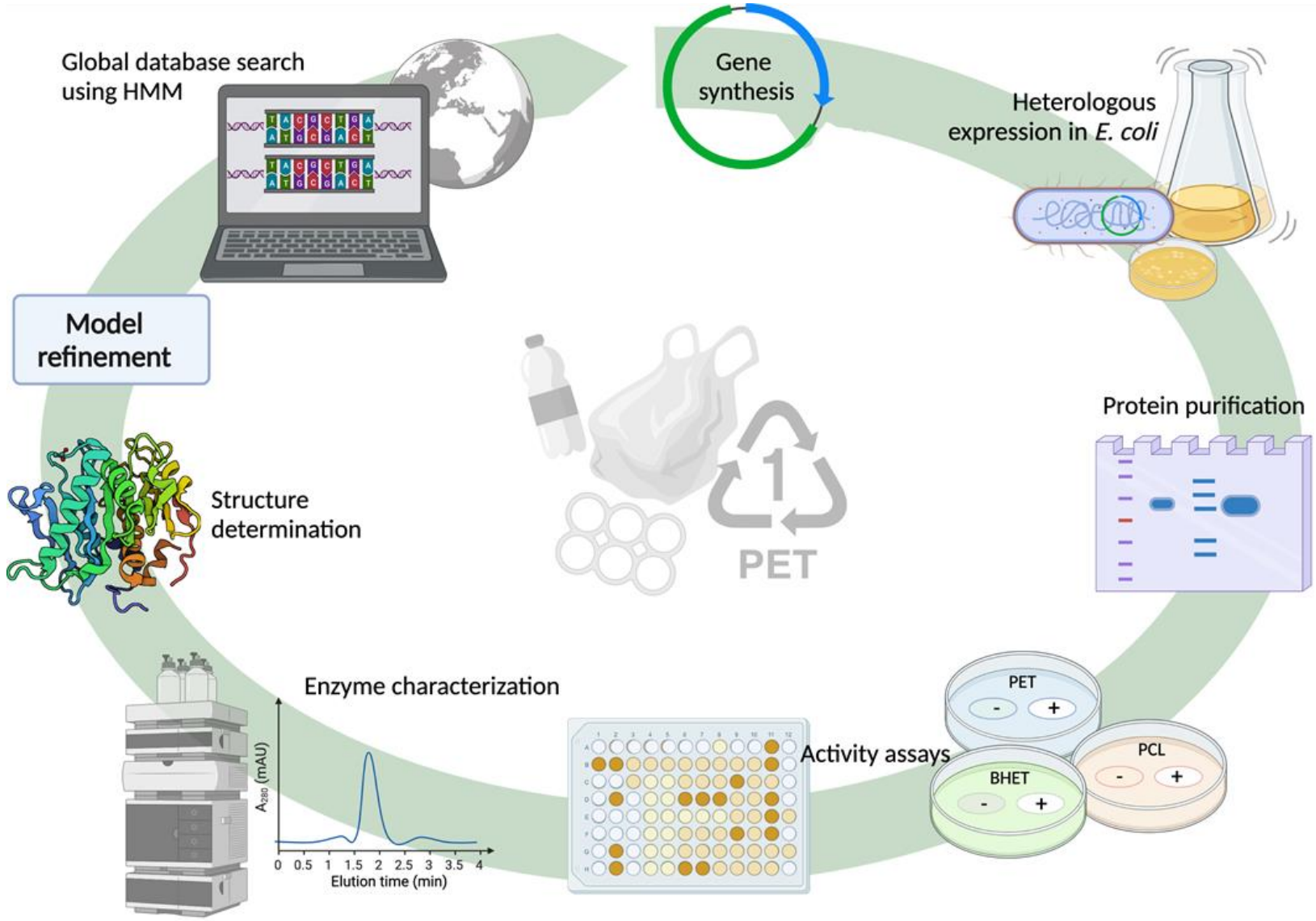
https://pazy.eu/



In collaboration with AG Jürgen Pleiss

Universität Stuttgart
Institut für Biochemie und Technische Biochemie

How to find new PET degrading enzymes



Chow J., Pérez-García P., Dierkes R., Zhang H., and Streit W.R. *Methods in Metagenomics*, 3rd Ed. in press

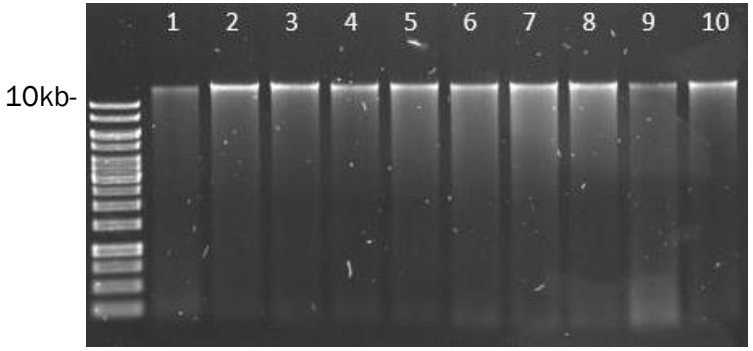
Finding plastic eating bacteria: Metagenomics

Global Metagenome Sampling

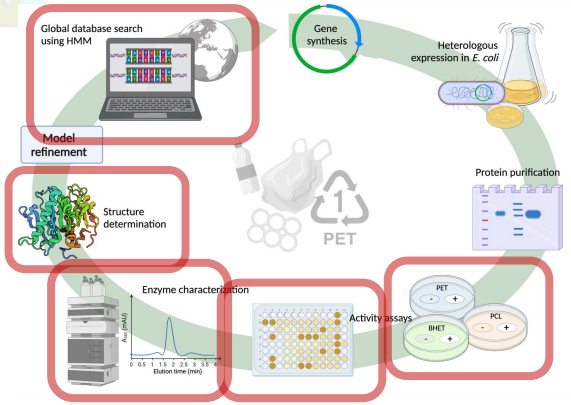
Metagenome DNA

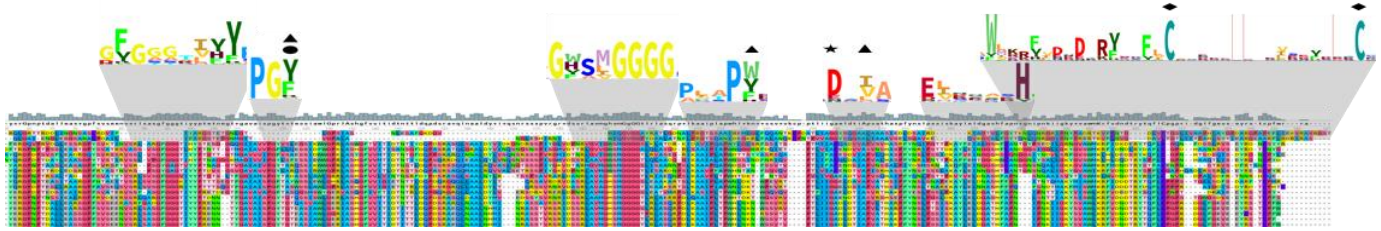
TIERPARK Hagenbeck

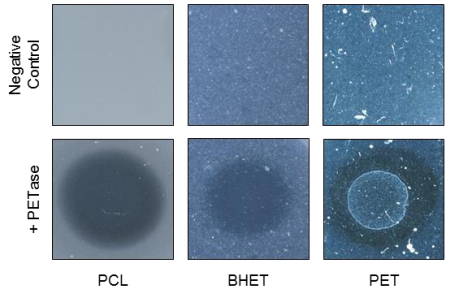
Environmental DNA samples



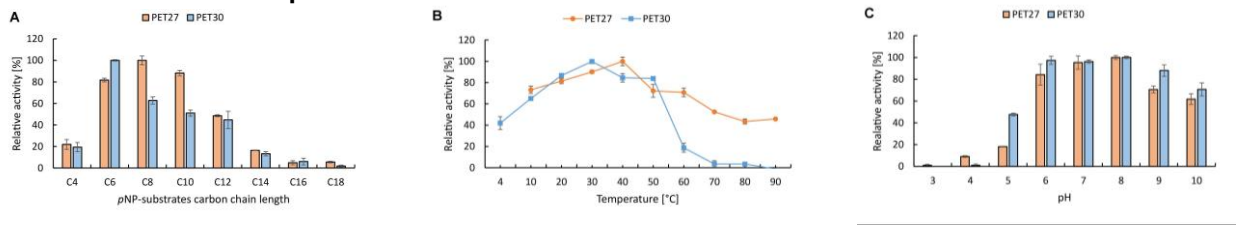
How to find new PET-degrading enzymes

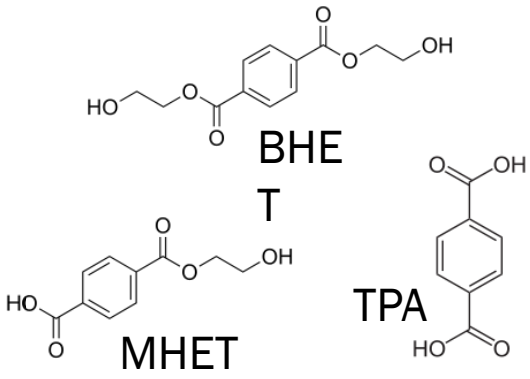


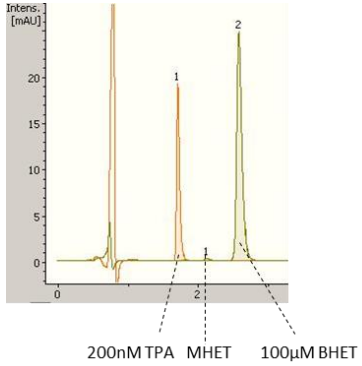


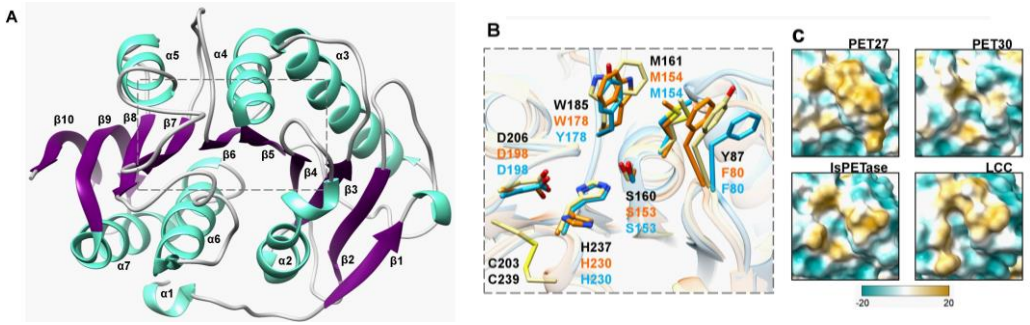


Optimization of reaction conditions:



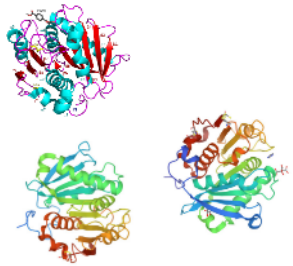




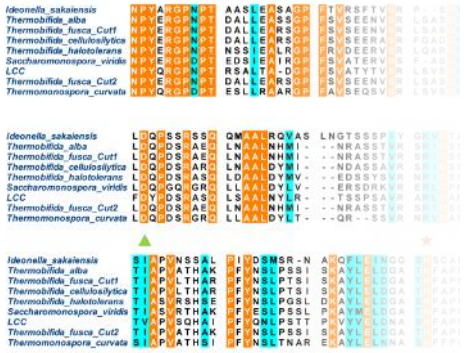


Finding plastic eating bacteria: HMM screening

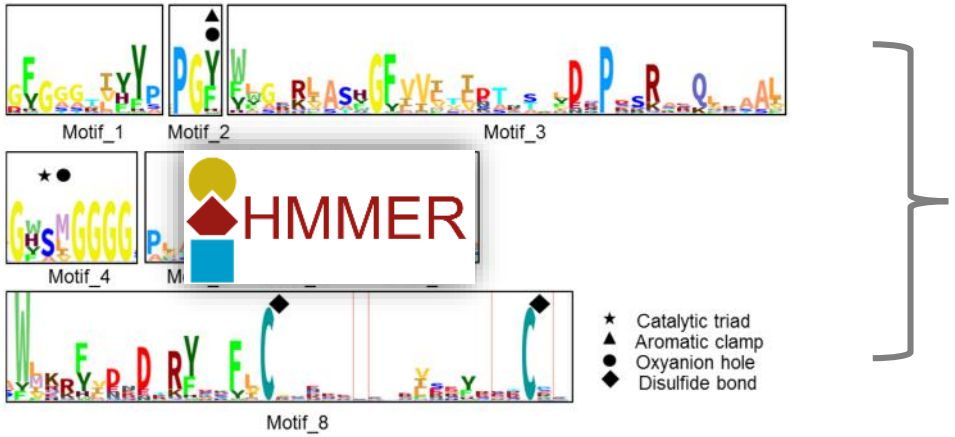
Known enzymes
With desired
function

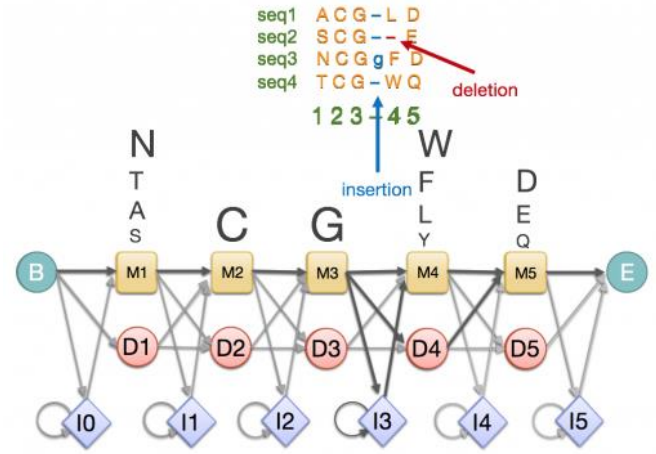


Multiple sequence
alignment



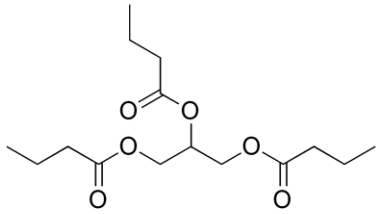
Hidden Markov Model construction and
search



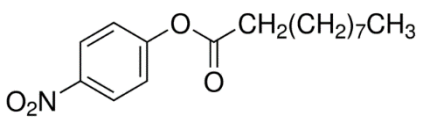


PET esterase screening substrates

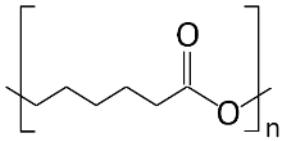
TBT
Tributyrin



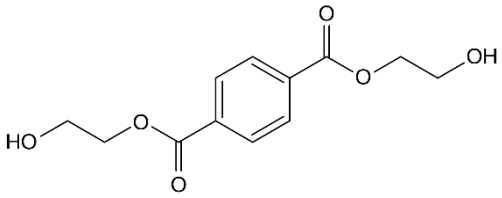
pNP-esters
Para Nitrophenol – esters



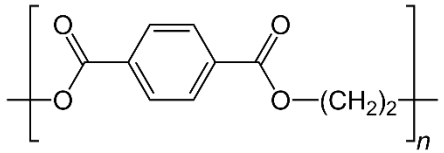
PCL
Polycaprolactone



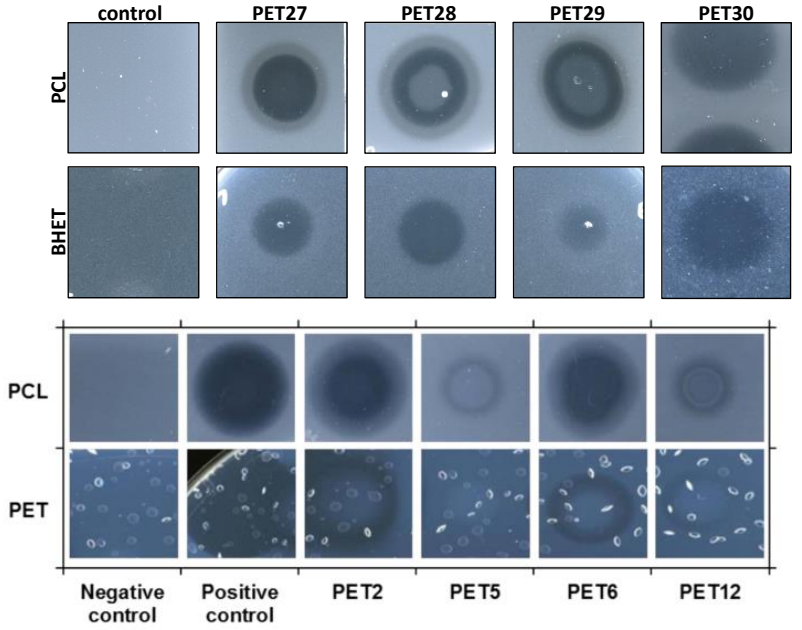
BHET
Bis(2-Hydroxyethyl) terephtalate



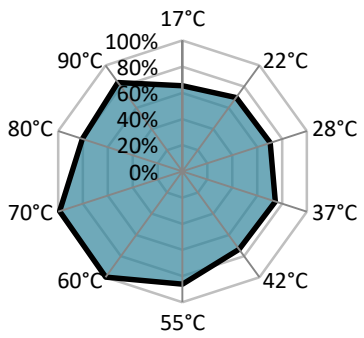
PET Film
PET Powder
Polyethylene terephtalate



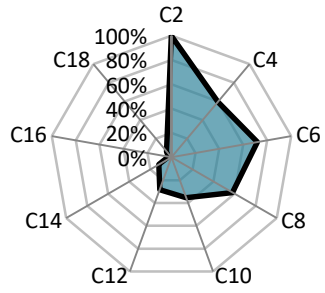
Agar clearing assays



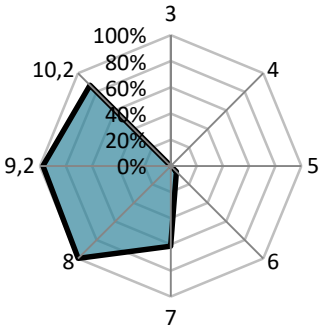
pNP-ester degradation



Temperature



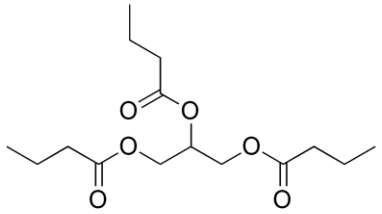
Substrate chain length



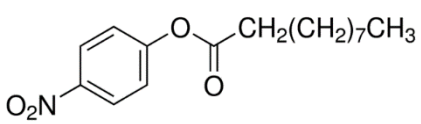
pH

PET esterase screening substrates

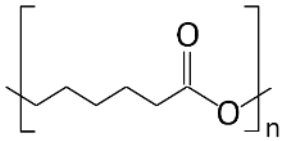
TBT
Tributyrin



pNP-esters
Para Nitrophenol – esters



PCL
Polycaprolactone



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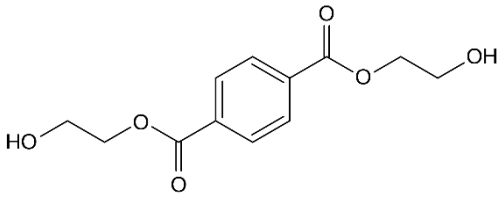
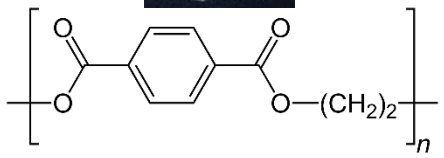
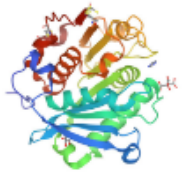
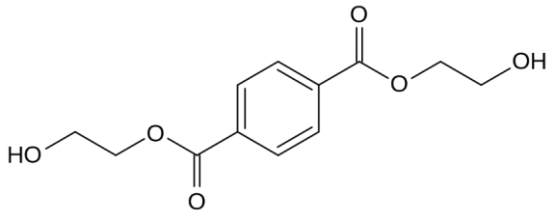


PET Film
PET Powder
Polyethylene terephtalate

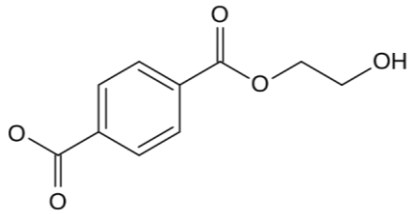




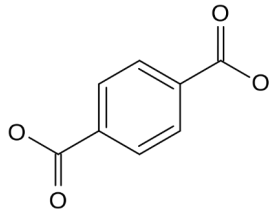
Degradation



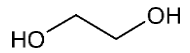
BHET
Bis(2-Hydroxyethyl) terephtalate



MHET
Mono(2-Hydroxyethyl) terephtalate

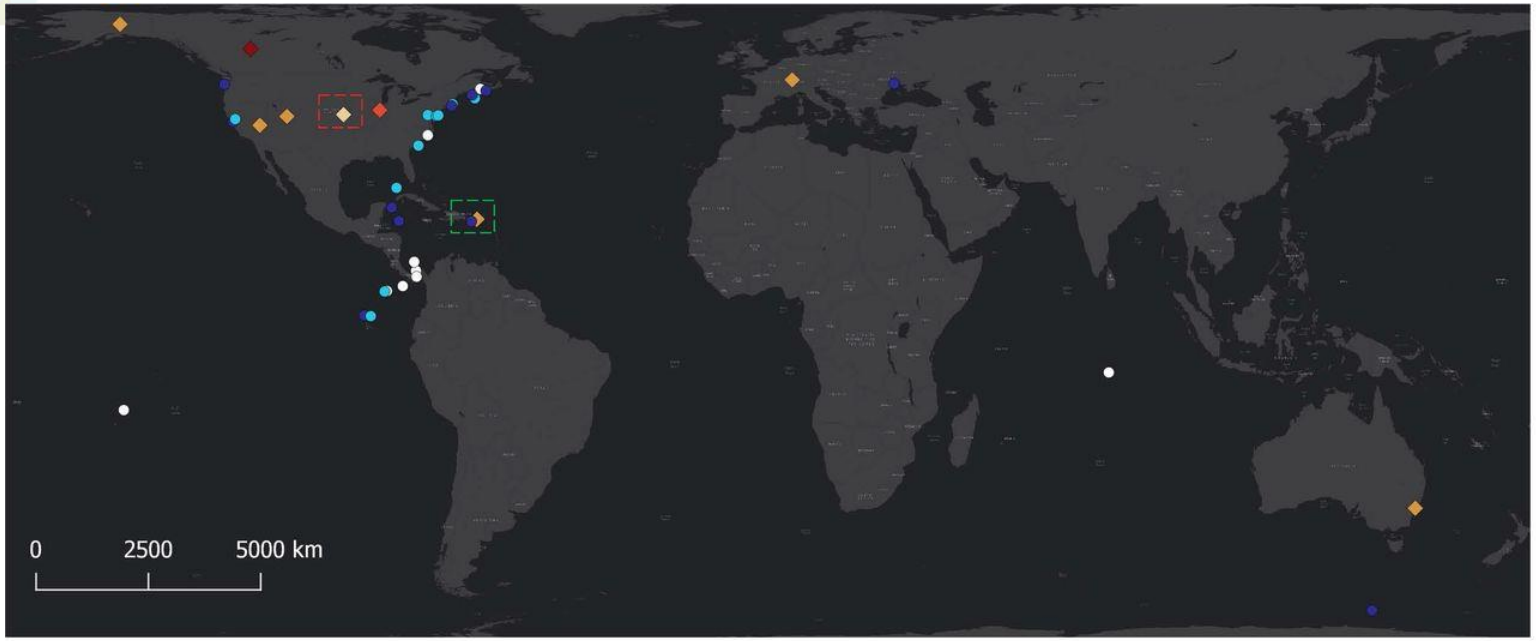


TPA
Terephtalic acid



EG
Ethylene glycol

PET degrading enzymes are hard to find

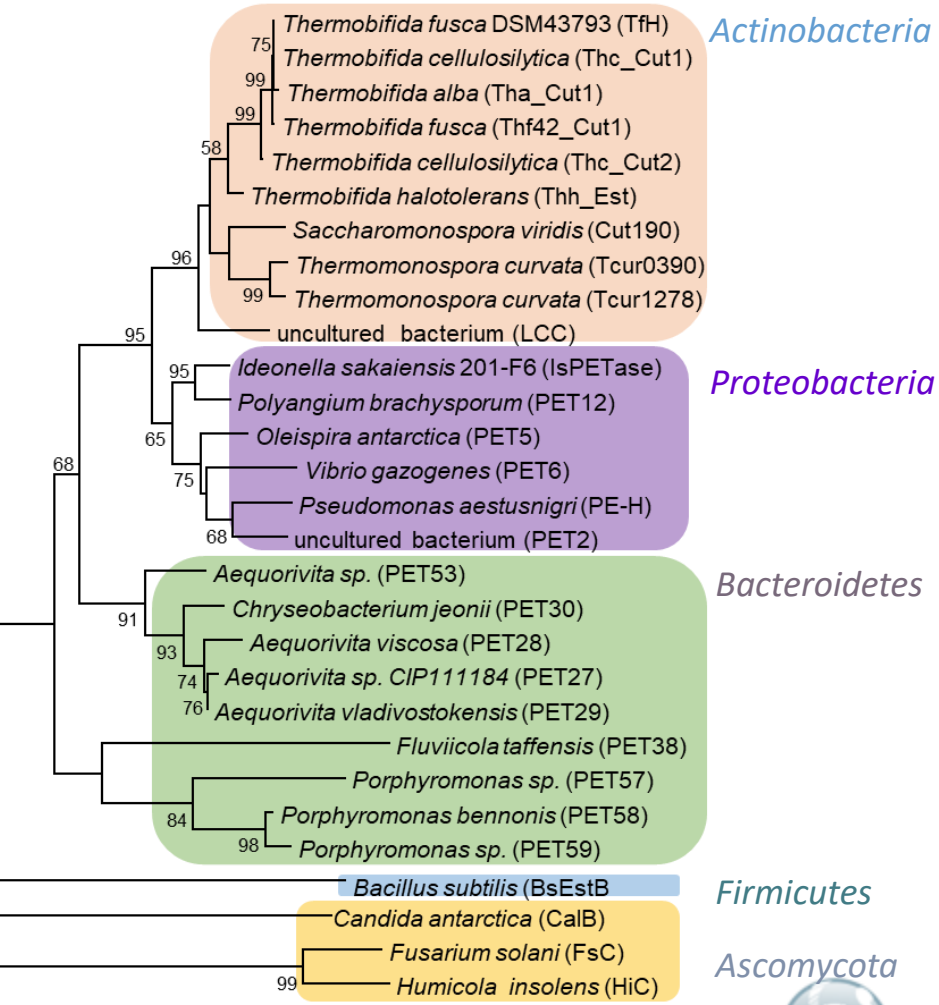


Frequency of potential PET hydrolases in hits/Mb

Marine [31]
- 0.004 - 0.03 [9]
- 0.03 - 0.05 [11]
- 0.05 - 1 [11]

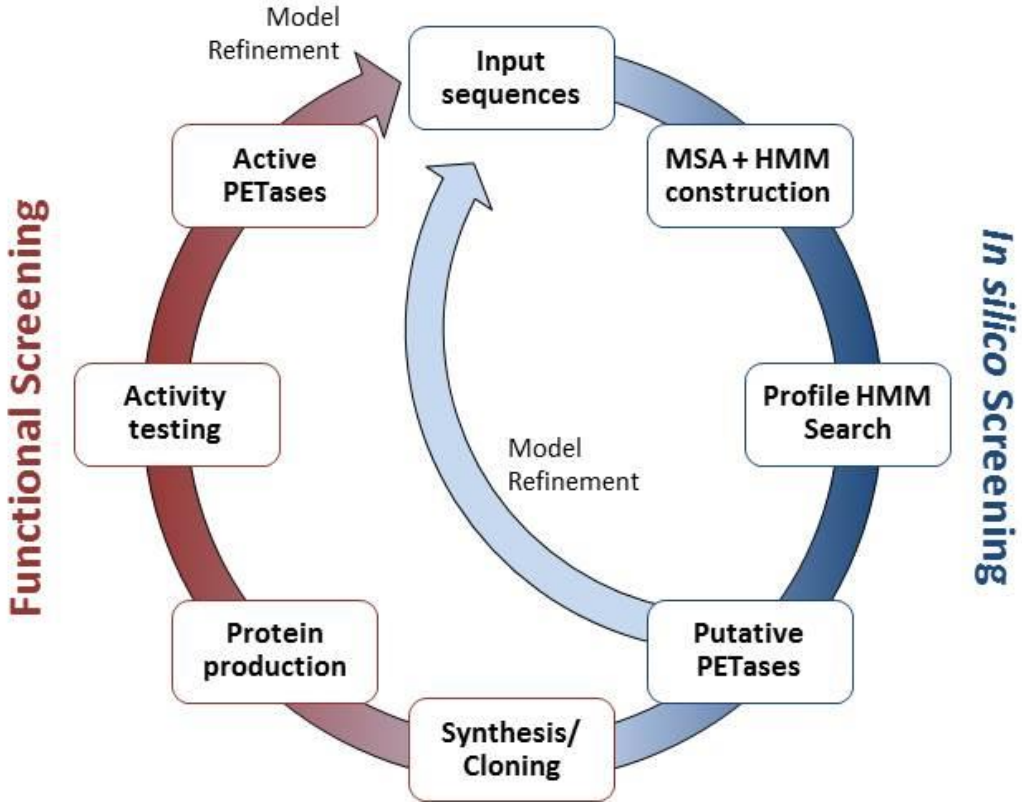
Terrestrial [11]
- 0.0001 - 0.01 [3]
- 0.01 - 0.05 [6]
- 0.05 - 1 [1]
- ≥ 1.5 [1]

29 PETases in 5 phylogenetic clusters

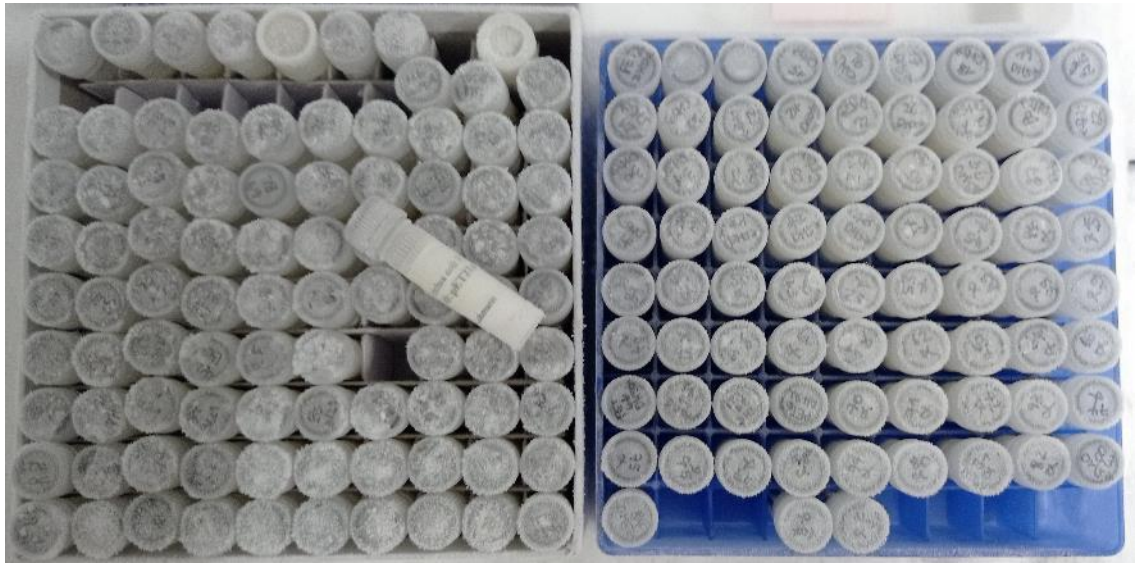


Danso et al., 2018; Zhang et al., 2022

Finding plastic eating bacteria: An ongoing search…

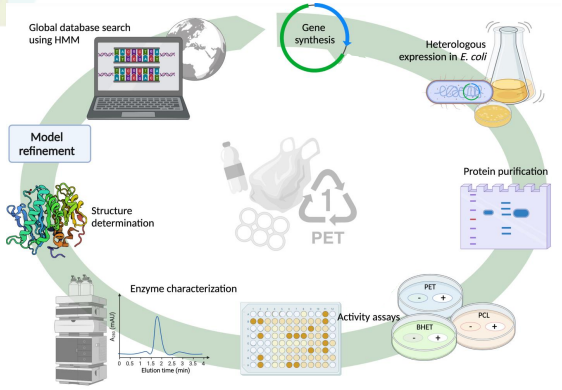


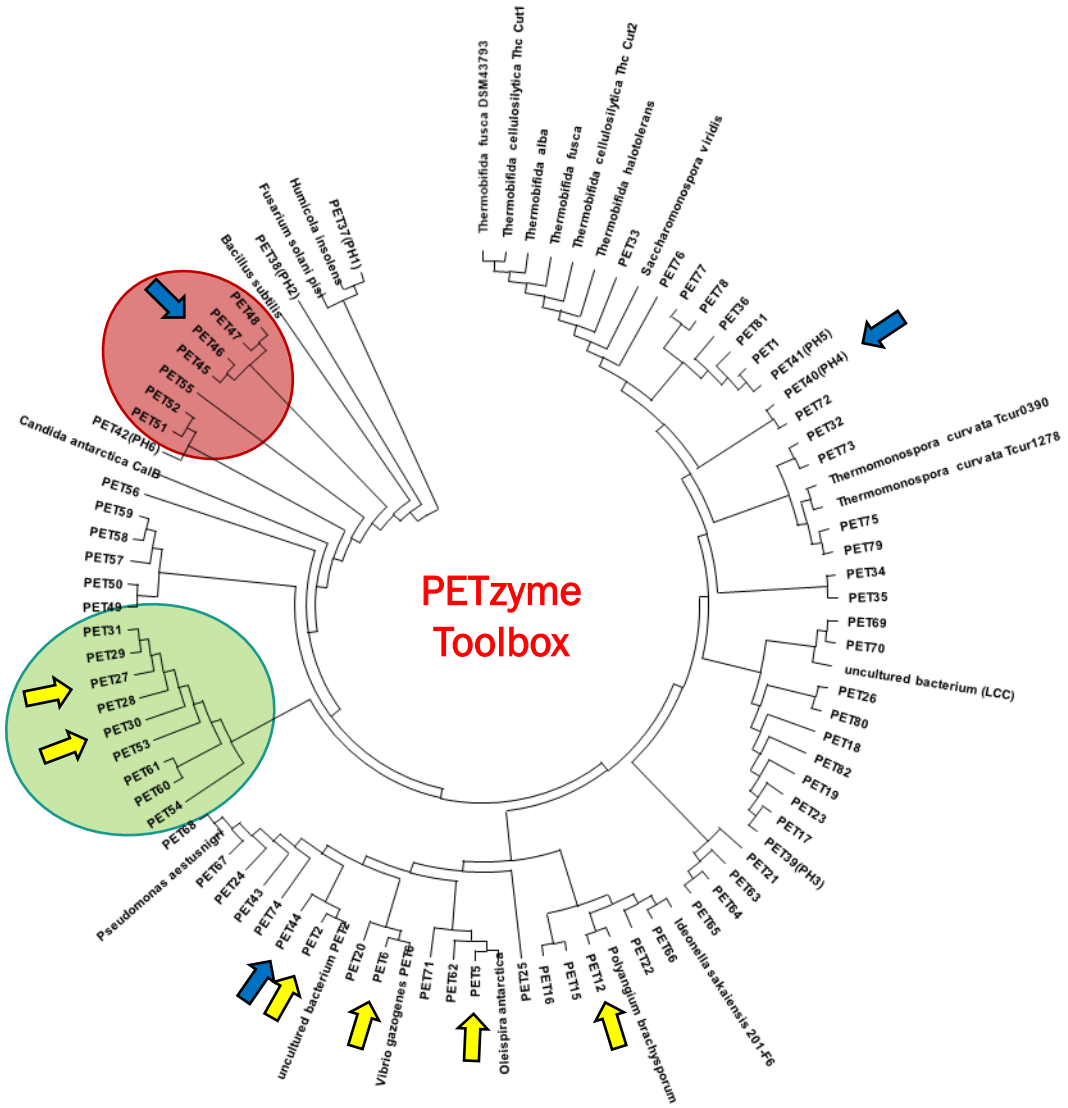
Until now: Synthesized a collection of > 80 different potential and verified PETase genes



Pérez-Garcia et al. 2021

PETzyme: A toolbox of microbial PET-degrading enzymes





Published PETases: 40

PETzyme toolbox: **80+**

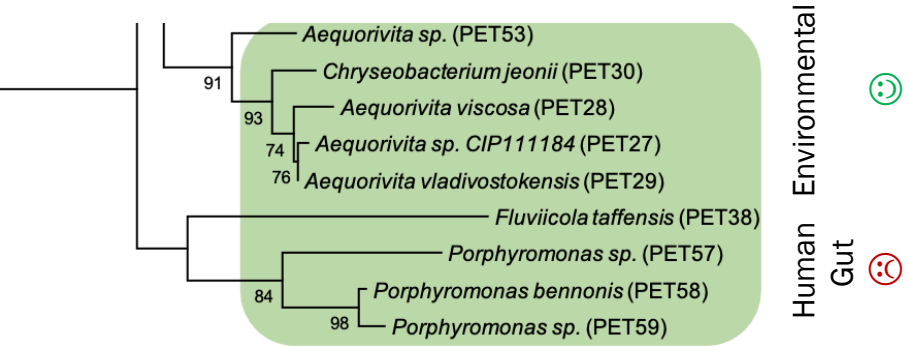
Published
Soon to be submitted

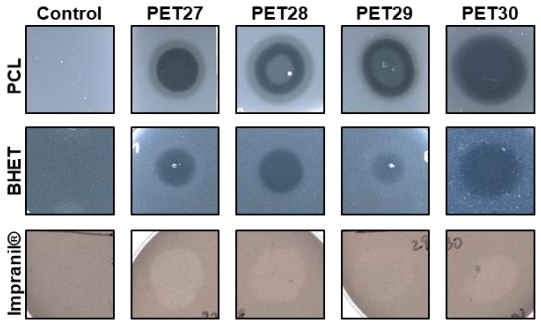
PET2, 5, 6, 12: Danso, D., Schmeisser, C., Chow, J., Zimmermann, W., Wei, R., ...and Streit, W.R. (2018) Appl Environ Microbiol 84

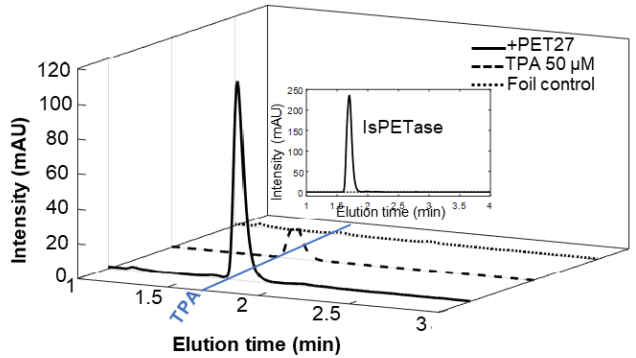
PET27-31: Zhang, H., Perez-Garcia, P., Dierkes, R.F., Applegate, V., ...Pleiss, J., ...Smits, S.H.J., Chow, J., and Streit, W.R. (2022) Front Microbiol 12: 803896

PET6: Weigert, S., Perez-Garcia, P.,... Chow, J., Streit, W.R. and Höcker, B. (2022) Proteins, under review

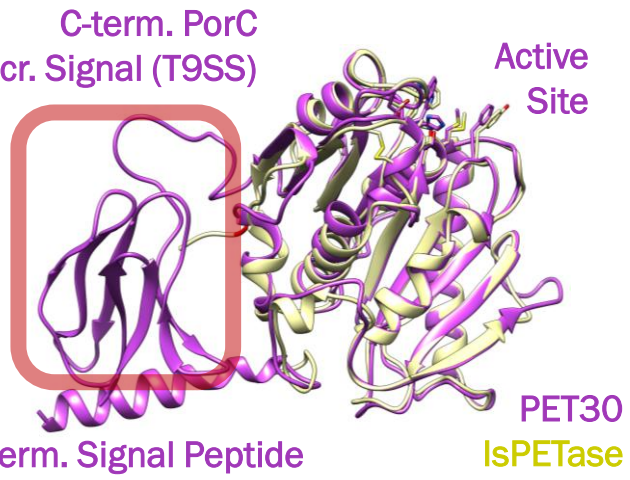
The first bacteroidetal PET-degrading enzymes

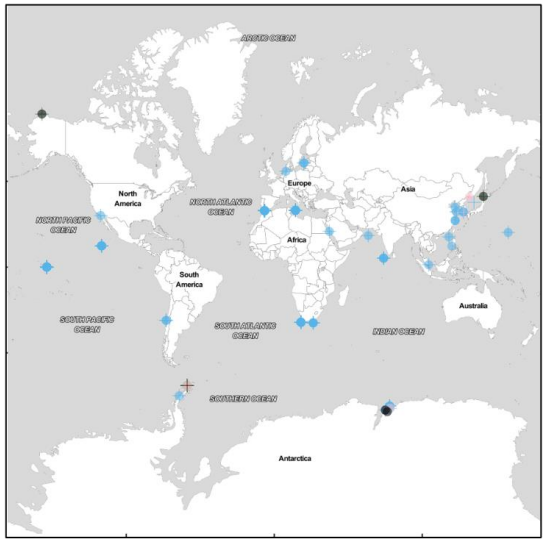






PDB 7PZJ

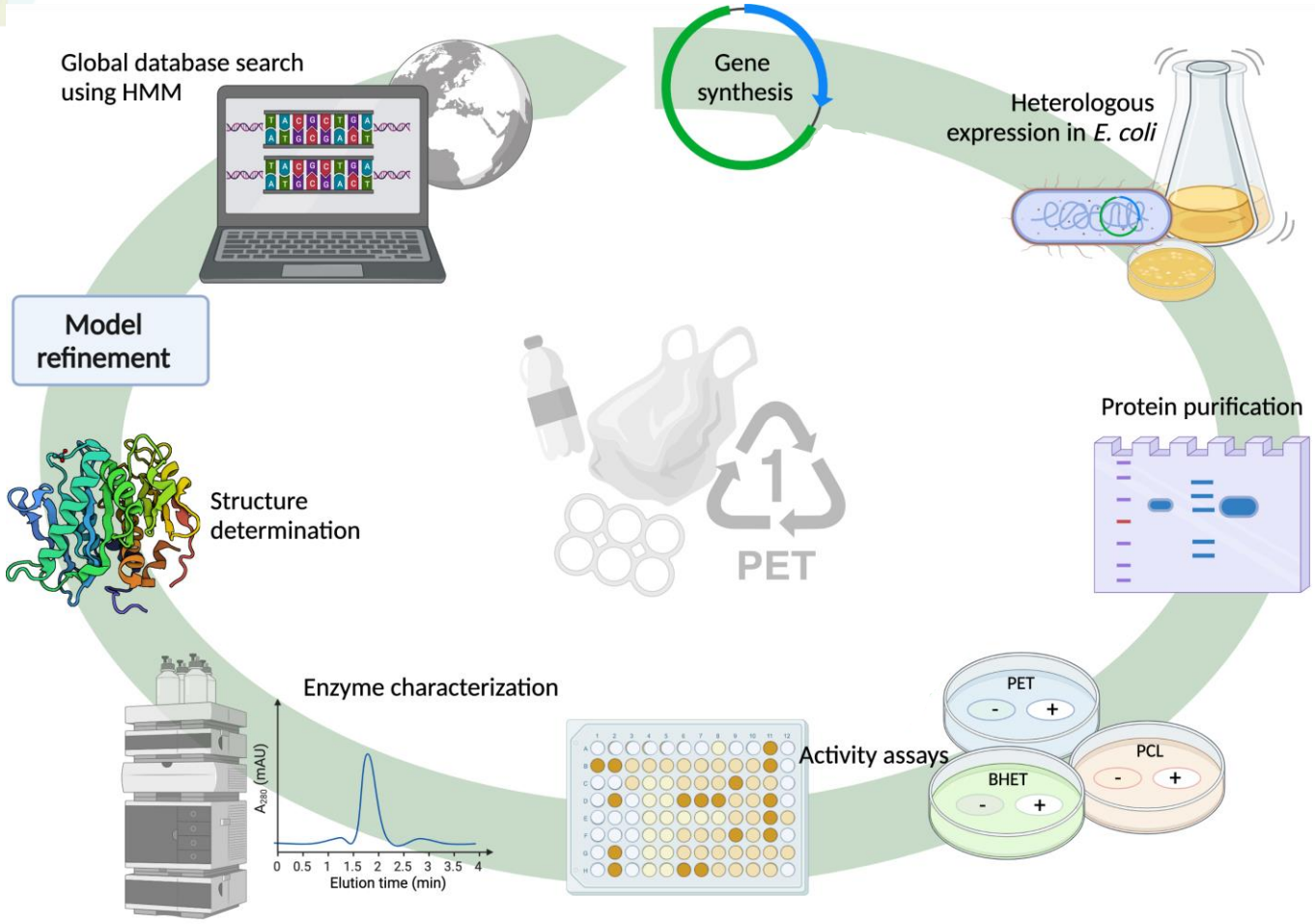




Bacteroidetal PETases are spread worldwide

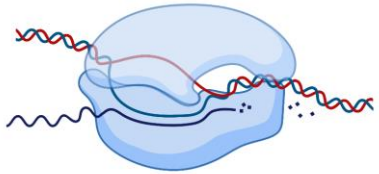
Zhang, H., Perez-Garcia, P., Dierkes, R.F., Applegate, V., ...Pleiss, J., ...Smits, S.H.J., Chow, J., and Streit, W.R. (2022) *Front Microbiol* **12**: 803896

In vitro transcription and translation of PETases



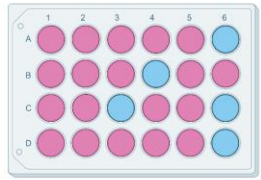
PCR of candidate genes

In vitro transcription



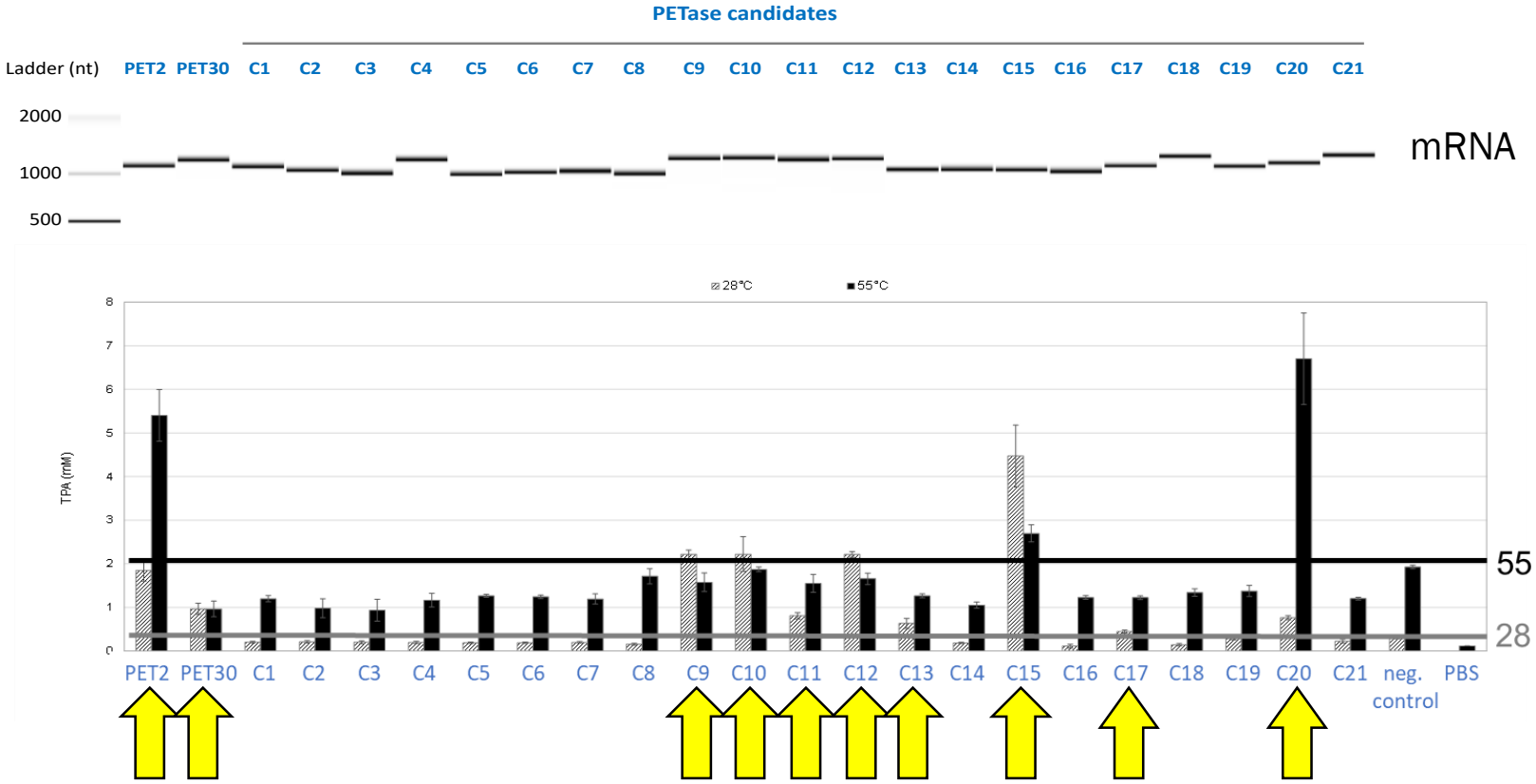
In vitro translation

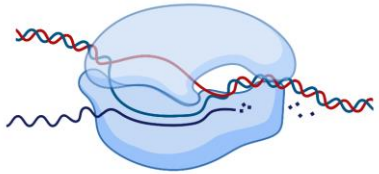
Activity assay

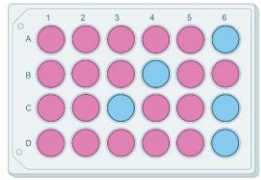


Han Y., Kinfu B., ..., Perez-Garcia, P., ..., Schwaneberg U., Chow J., Werner F. and Streit, W.R. (2022) *under review*

In vitro transcription and translation of PETases

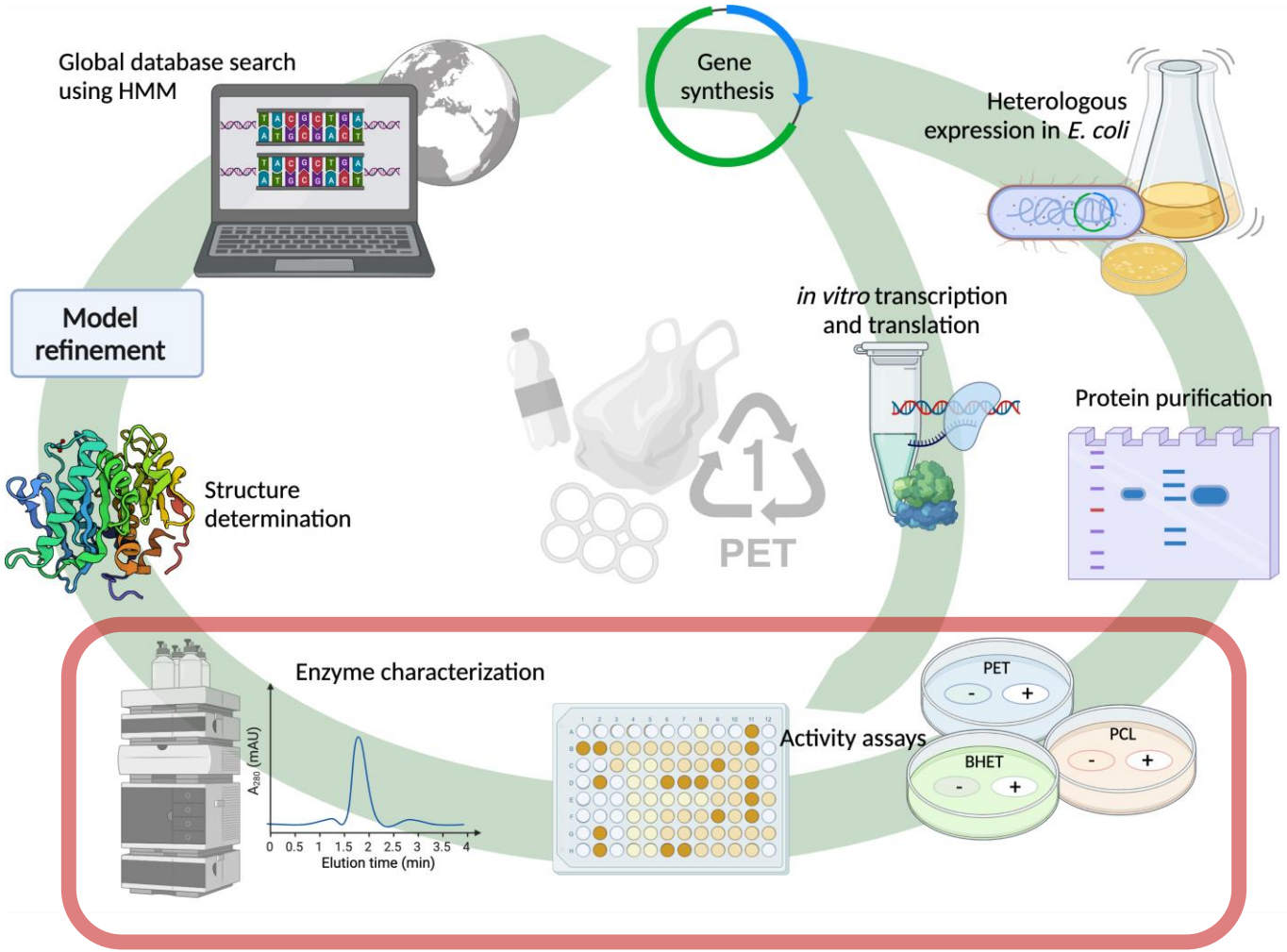






Han Y., Kinfu B., ..., Perez-Garcia, P., ..., Schwaneberg U., Chow J., Werner F. and Streit, W.R. (2022) *under review*

Fluorescent reporter strain for fast activity measurements

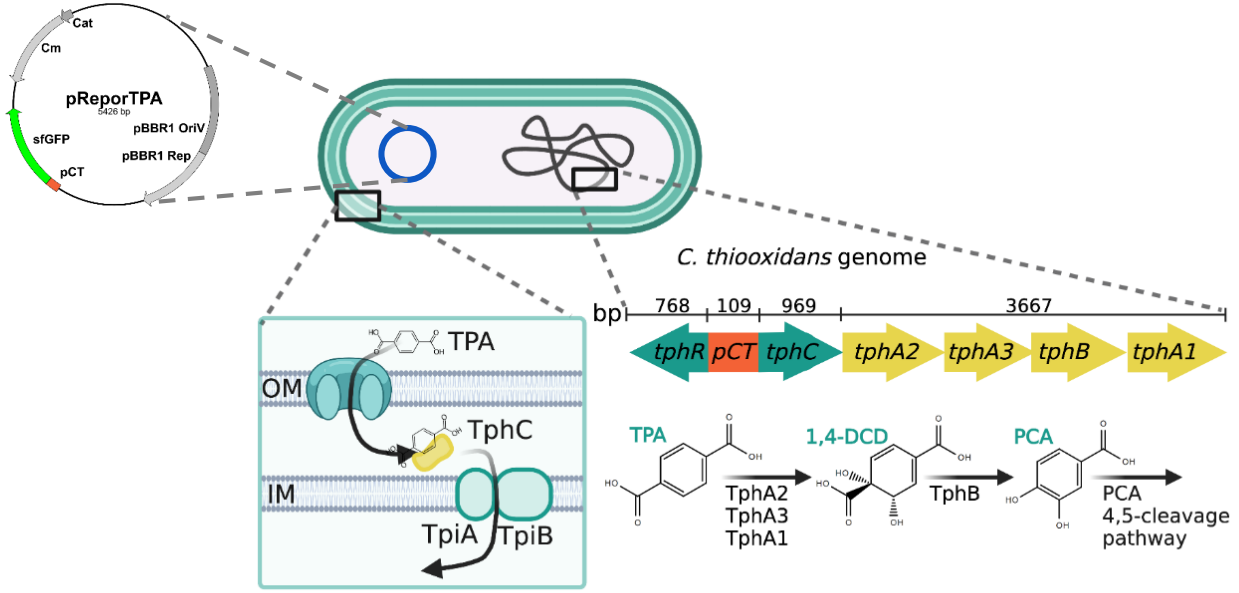


ReporTPA™: A fast and sensitive TPA reporter

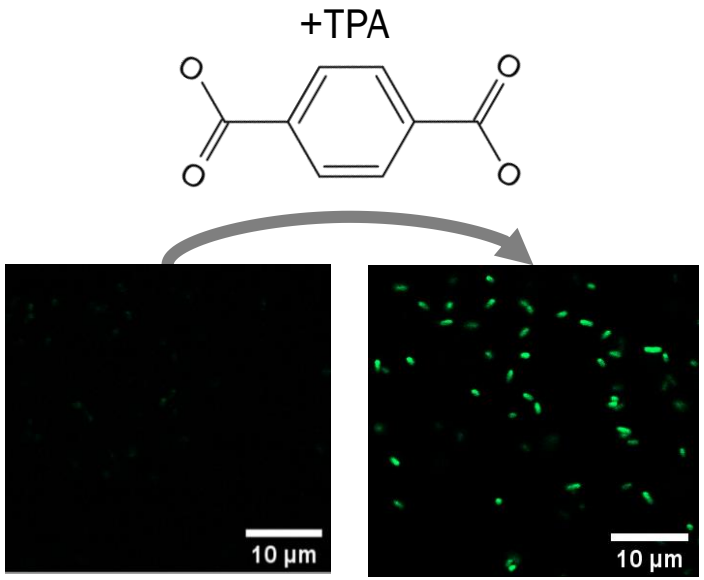
Reporter strain based on *Comamonas thioxidans*

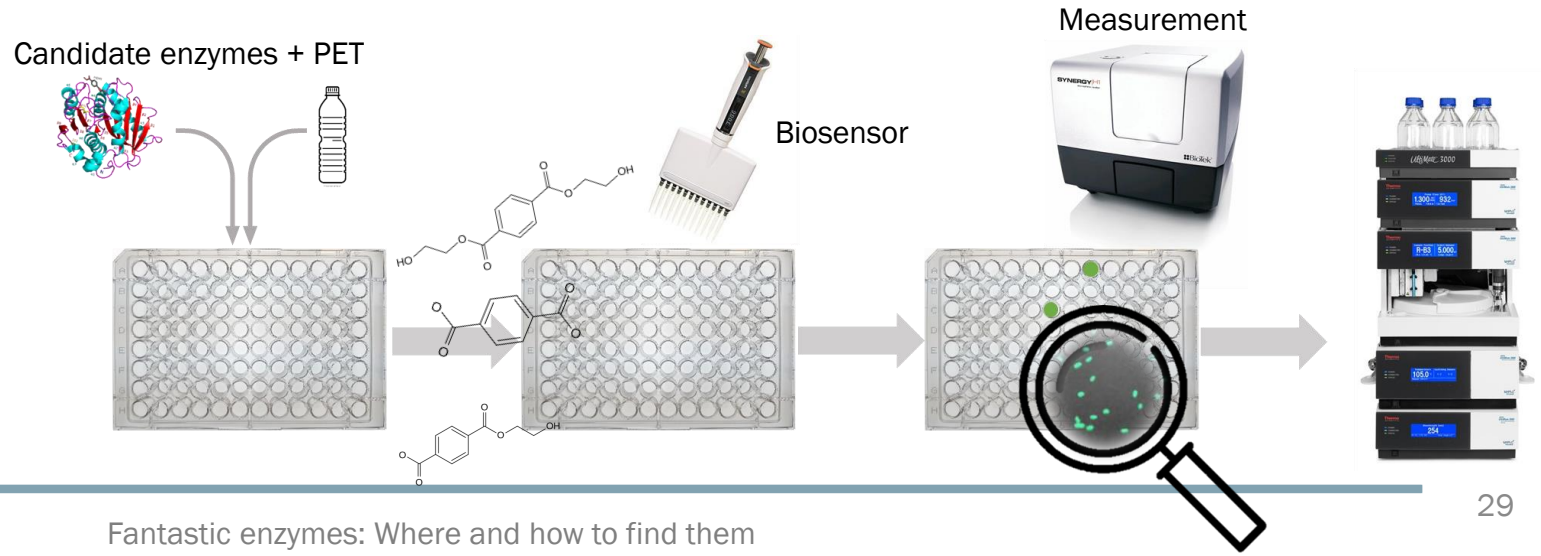
Contains gene cluster for TPA catabolism

TPA-degrading capability is used as starting point for Reporter



Dierkes et al., 2022 - Manuscript in preparation





Conclusions

- Microbes harbour an enormous potential on biocatalysts due to their metabolic versatility
- This potential can be accessed by metagenomics
- In bioindustries, hydrolases and esp. lipases and carboxylesterases have one of the highest shares
- UHAM built up an enzyme collection of >80 lipases by functional screenings
- With a sequence-based approach, >80 potential PET degrading enzymes have been found
- At UHAM, innovative tools are developed that help to fasten the discovery of novel enzymes: *In vitro* expression system and reporter strain for TPA detection

Team in Hamburg

External collaborators

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Rolf Daniel, Göttingen, Germany

Ruth Schmitz-Streit, CAU Kiel, Germany

Alex Almeida, Sangre Centre; Hinxton UK

& others....

Federal Ministry of Education and Research

DFG Deutsche Forschungsgemeinschaft

RAHN-QUADE-STIFTUNG

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...and thank you for your attention!


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Alan Wypych


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PLASTISEA

FUTURENZYME

LipoBiocat

DAAD

FUTURENZYME