RAPID™ System

Microbial Ecology

2024/2025

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What is it?

The **RapID system** is a qualitative method designed for the identification of bacteria and yeasts.

It utilizes enzymatic and non-enzymatic reactions to provide quick and accurate results, significantly reducing time.

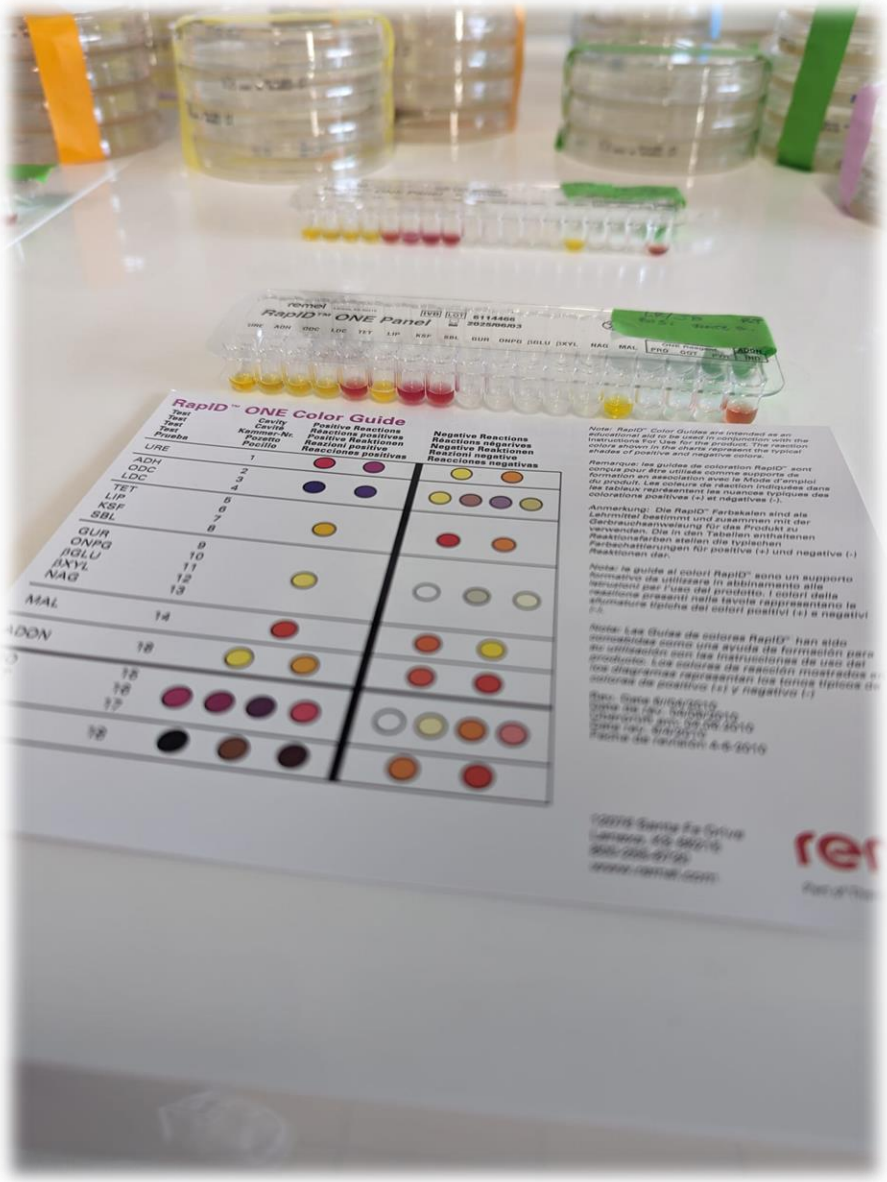
Every RapID system is suitable for the identification of different bacteria

RapID™ ONE

- RapID™ ONE System utilizes diagnostic panels that are disposable plastic trays with 18 reaction cavities containing dehydrated reactants.
- Assessment based of 19 different enzyme and metabolic reactions
- RapID™ ONE is indicated for Enterobacteriaceae, Gram-negative and oxidase-negative bacteria.
- It can detect up to 70 bacteria

How does it work?

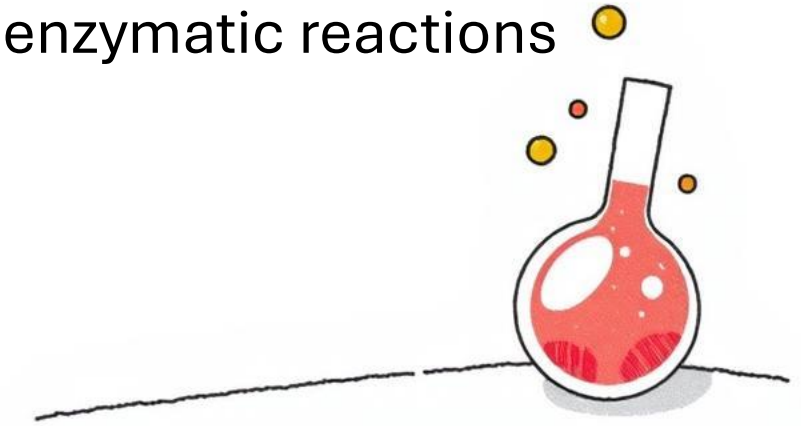
- Inoculum preparation with utilization of pure culture
- Inoculation in the panel respecting McFarland standards
- The system employs enzymatic and non-enzymatic reactions. The interaction with the substrate produces either acidic or basic byproducts that alter the pH of the medium.
- This change in pH triggers a color change in the pH indicator, chromogenic compounds or sensitive dyes present in the panel.



Enzymes in RapID™ System

- Hydrolases
- Deaminases
- Decarboxylases
- Proteases
- Phosphatases
- Oxidases
- Reductases
- Esterases and lipases
- Fermentation/Dissimilation enzymes
- Other metabolic enzymes

RapID™ ONE System allows the assessment of 19 enzymatic reactions



Enzymes in RapID™ ONE

Urease (URE)
Arginine Dihydrolase (ADH)
Lysine Decarboxylase (LDC)
Ornithine Decarboxylase (ODC)
Phenylalanine Deaminase (PHE)
Beta-Galactosidase (ONPG)
Beta-Xylosidase (XYL)
Alpha-Galactosidase (GAL)
Beta-Glucuronidase (GLU)
Beta-N-Acetylglucosaminidase (NAG)
Beta-Glucosidase (BGL)
Beta-Fucosidase (FUC)
Carbohydrate-specific hydrolases (7)

Urease (URE)

Urease is an enzyme that catalyzes the hydrolysis of urea, breaking it down into ammonia and carbon dioxide. This reaction elevates the pH level, leading to a color change indicating the presence of urease activity.

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

[1]

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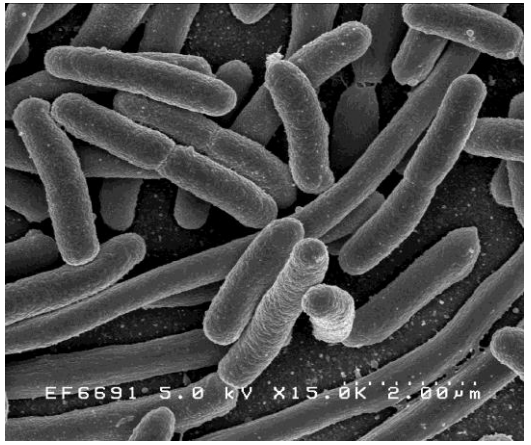

[2]

Beta-Glucuronidase (GLU)

This enzyme catalyzes the hydrolysis of glucuronides, breaking them down into glucuronic acid and p-nitrophenol, a chromogenic compound. This reaction, in alkaline conditions, produces a yellow color in the well, indicating the presence of beta-glucuronidase activity. This enzyme is commonly used to identify bacteria like *Escherichia coli*.

(Fishman, W. H. (1974). β-Glucuronidase. In *Methods of enzymatic analysis* (pp. 929-943). Academic Press.)

Enzymes in RapID™ ONE

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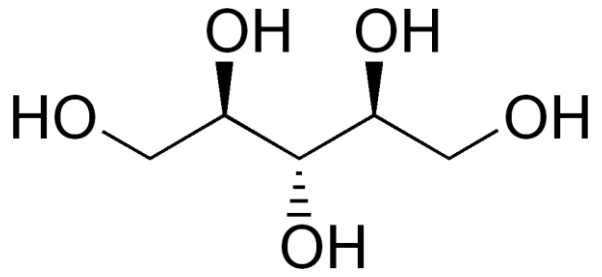

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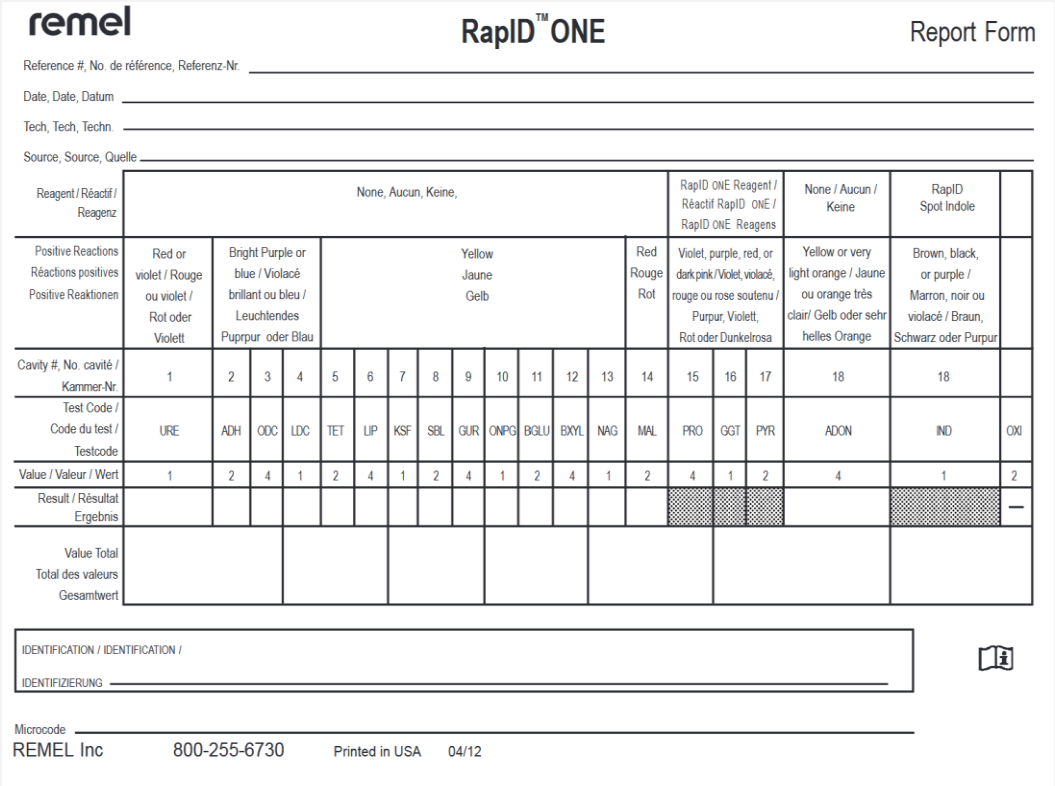
Adonitol Dehydrogenase (ADON)

Adonitol Dehydrogenase is an enzyme that catalyzes the metabolism of adonitol, a sugar alcohol, converting it into its corresponding acid. This reaction produces intermediates that lead to a color change indicating the presence of adonitol-degrading activity. This test is useful for identifying certain Gram-negative bacteria based on their carbohydrate metabolism profiles.

How to obtain results?

- The outcomes of the wells are scored as positive (+) or negative (–) based on the observed color.
- Each microorganism has a unique sequence based on the reaction outcomes
- Comparison with a database (RapID ERIC™ software) or scoring system
- The software provides the species or the similarity with the species



remel — RapID™ ONE — Report Form

Reference #, No. de référence, Referenz-Nr. _____

Date, Date, Datum _____

Tech, Tech, Techn. _____

Source, Source, Quelle _____

Reagent / Réactif / Reagenz	None, Aucun, Keine,													RapID ONE Reagent / Réactif RapID ONE / RapID ONE Reagens			None / Aucun / Keine	RapID Spot Indole		
Positive Reactions Réactions positives Positive Reaktionen	Red or violet / Rouge ou violet / Rot oder Violett	Bright Purple or blue / Violacé brillant ou bleu / Leuchtendes Pupprpur oder Blau			Yellow Jaune Gelb								Red Rouge Rot	Violet, purple, red, or dark pink / Violet, violacé, rouge ou rose soutenu / Purpur, Violett, Rot oder Dunkelrosa			Yellow or very light orange / Jaune ou orange très clair/ Gelb oder sehr helles Orange	Brown, black, or purple / Marron, noir ou violacé / Braun, Schwarz oder Purpur		
Cavity #, No. cavité / Kammer-Nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	18	
Test Code / Code du test / Testcode	URE	ADH	ODC	LDC	TET	LIP	KSF	SBL	GUR	ONPG	BGLU	BXYL	NAG	MAL	PRO	GGT	PYR	ADON	IND	OXI
Value / Valeur / Wert	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
Result / Résultat Ergebnis																				–
Value Total Total des valeurs Gesamtwert																				

IDENTIFICATION / IDENTIFICATION / IDENTIFIZIERUNG _____

Microcode _____

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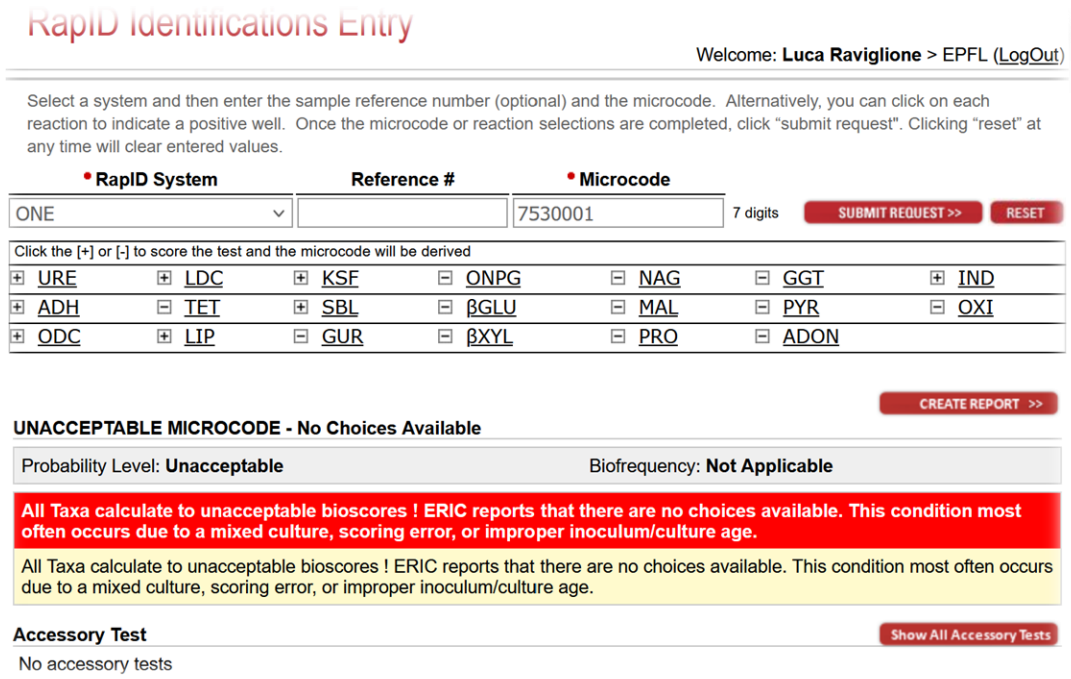
Considerations and limitations

- Importance of Using Pure Cultures → Mixed microbial populations can lead to inaccurate identification
- Limited database of species
- Ambiguity in color interpretation



Presentation of science paper

SOURCE : Zurier, Hannah S., and Julie M. Goddard. "A high-throughput expression and screening platform for applications-driven PETase engineering." *Biotechnology and Bioengineering* 120.4 (2023): 1000-1014.

Received: 27 September 2022 | Revised: 20 December 2022 | Accepted: 26 December 2022

DOI: 10.1002/bit.28319

ARTICLE

BIOTECHNOLOGY BIOENGINEERING WILEY

A high-throughput expression and screening platform for applications-driven PETase engineering

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U.S. Department of Agriculture

Abstract

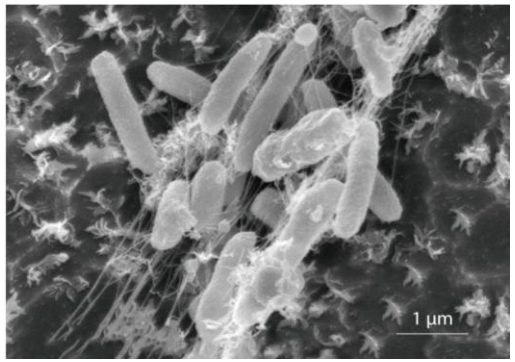
The environmental consequences of plastic waste have impacted all kingdoms of life in terrestrial and aquatic ecosystems. However, as the burden of plastic pollution has increased, microbes have evolved to utilize anthropogenic polymers as nutrient sources. Of depolymerase enzymes, the best characterized is PETase, which hydrolyzes aromatic polyesters. PETase engineering has made impressive progress in recent years; however, further optimization of engineered PETase toward industrial application has been limited by lower throughput techniques used in protein purification and activity detection. Here,

Presentation of science paper

<u>Goal of the study</u> : development and testing of new PETase enzyme variants

- PETase : enzyme breaking down PET plastic (polyethylenepterephtalate) into soluble monomers of MHET (mono-2-hydroxyethylterephtalate)
- PETase is naturally produced by the bacteria *Ideonella sakaiensis*, discovered in Japan in 2016

Bacteria Ideonella sakaiensis



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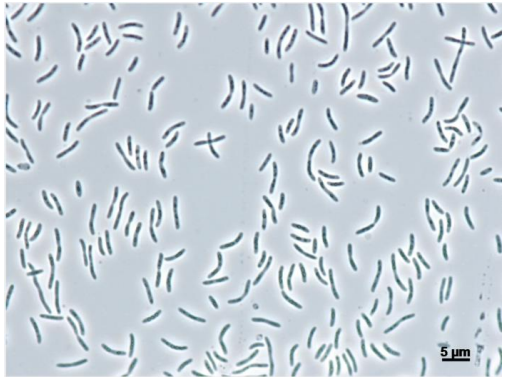

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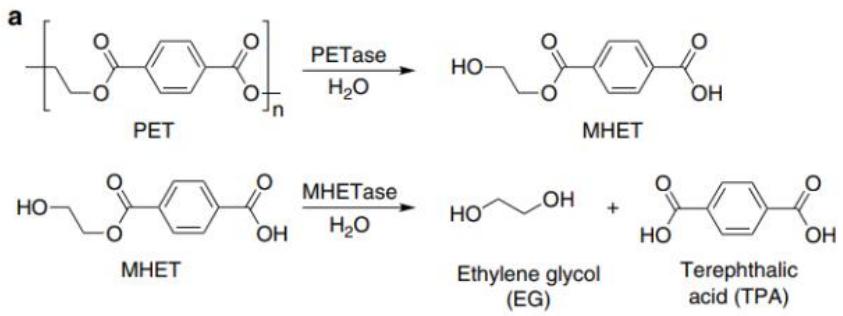

PET biodegradation pathway by Ideonella sakaiensis [7]

Presentation of science paper

Why looking for new PETase variants ?

- increase PETase activity for faster PET degradation
- increase PETase overall stability

How are these new variants made ?

- Analyzed the *I. sakaiensis* PETase sequence using computational tools to identify potentially stable, functional mutations
- Creation of a mutant library of 57 mutations to test

➔ The **DNA sequence**, **incorporating the PETase mutations**, is **synthesized** to create **plasmids**, which are then **inserted into *E. coli*** bacteria for the **production of the mutant enzyme.**

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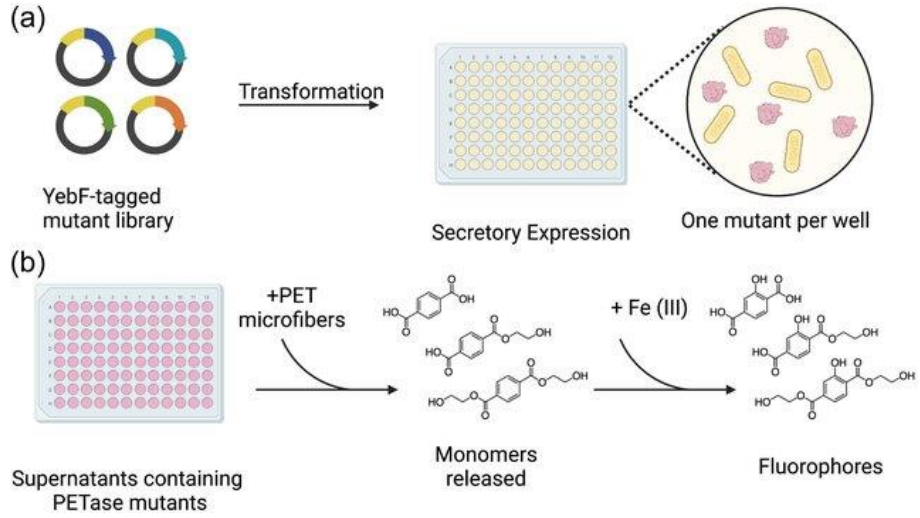
Enzyme Activity testing

- The engineered E.Coli were put into microwell plate for the mutant PETase expression
- **PET microfibers** is then inserted into microwell plate containing the different mutant **PETase expressions**
- After 72h of incubation, a mixture of FeSO4/EDTA is added, which will react with the PET degradation product (MHET, BHET, TPA) and produce **fluorescence**
- Fluorescence is then measured using a spectrophotometer, with fluorescence intensity as a proxy to **estimate how much PET degradation products were formed**



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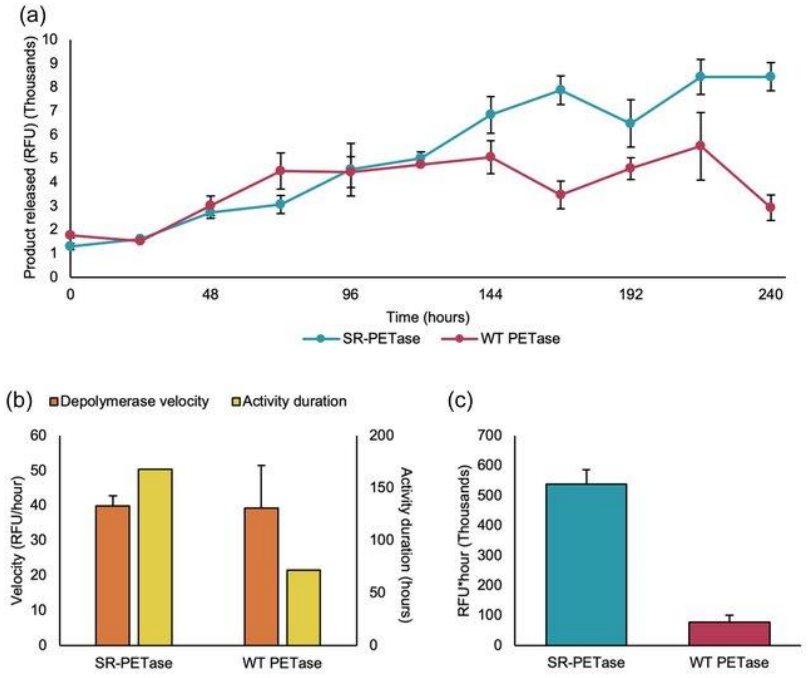
Results of study

[9]

- 12 PETase mutants had higher activity than wild type PETase
  → due overall **enhanced enzyme stability**
- After combining mutations
  → Selection of one **new PETase variant « SR-PETase »**
- SR-PETase : between **1.9-fold higher fluorescence** and **7.4-fold higher total product release x activity**
  (experiment over 10 days at 40°C)



SR-PETase outperforms WT-PETase across the range of activity and stability metrics

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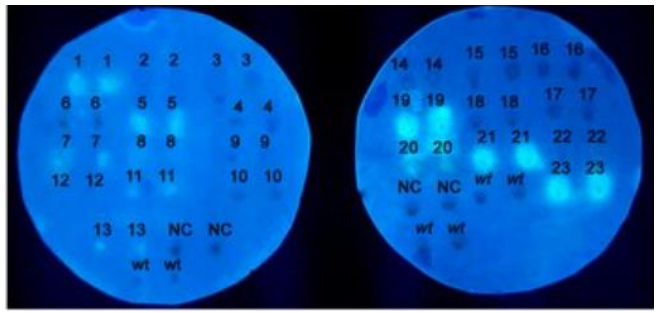
Differences of the tests :

- **RapID** : identify bacteria based on different enzymes presence
- **Study** : known bacteria and comparison of the variants of one enzyme

Similarities of the tests :

- Enzymatic activity testing
- Rapid test (4h vs ~72h)
- Visual results
  (fluorescence vs colorimetric)



[10]

[11]

Thank you for your attention
Questions?

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[2] https://it.wikipedia.org/wiki/Escherichia_coli
[3]https://en.wikipedia.org/wiki/Ribitol
[4] https://www.thermofisher.com/order/catalog/product/R8311006
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[8] Schematic depiction of high-throughput PETase engineering platform. (a) YebF-mediated PETase secretion enables near-parallel expression in microplates. Supernatants are directly analyzed for PETase activity without additional lysis or purification steps. Please note that the Escherichia coli cells and PEtase enzymes are not to scale in this graphic. (B) Ferric iron oxidation of PETase degradation products creates fluorophores that enable near-simultaneous activity quantification. (from reference article)
[9] SR-PETase outperforms WTPETase across the range of activity and stability metrics. (a) Comparison of WT- and SR-PETase across a 240 h time course. Values represent means of n = 3 determinations with error bars indicating standard deviation. (b) Depolymerase velocity and activity duration of mutants. Velocity is plotted as orange bars on the left axis, duration is plotted as yellow bars on the right axis. Error bars indicate standard error of the mean. (c) Total product released × activity duration of WT PETase and SR-PETase. Error bars indicates standard error of the mean. (from reference article)
[10] Duță, Horia, Alina Filip, Levente Csaba Nagy, Emma Zsófia Aletta Nagy, Róbert Tőtős, and László Csaba Bencze. "Toolbox for the structure-guided evolution of ferulic acid decarboxylase (FDC)." *Scientific Reports* 12, no. 1 (2022): 3347.
[11] https://www.fishersci.fi/shop/products/rapid-one-system/11494139