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

WP4 - Small-scale enzyme production and characterisation

M18 Online Executive Committee Meeting

Nov. 14, 2022

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

Deliverables

Work package number [9]	WP4	Lead beneficiary [10]	4 - UHAM
Work package title	Small-scale enzyme production and characterisation		
Start month	1	End month	40

Description of work and role of partners
WP4 - Small-scale enzyme production and characterisation [Months: 1-40] **UHAM**, CSIC, BANGOR, UDUS, IST ID, CNR, FHNW, Bio_Ch, EUCODIS We propose 6 Tasks. Task 4.1 Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production M2-M30 Task Lead Partner – UHAM Participants: CSIC, BANGOR, UDUS, CNR, EUC
Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30 Task Lead Partner – FHNW Participants: CSIC, EUC
Task 4.3 Production of enzymes from their natural hosts M2-M30 Task Lead Partner – CNR Participants: IST-ID, BIO_CH
Task 4.4 Enzyme characterisation for selecting those with manufacturers' specifications M2-M36 Task Lead Partner – UDUS Participants: BANGOR, CSIC, UHAM, FHNW, IST-ID, EUC
Task 4.5 Decision-making strategy for selecting lead enzyme candidates M6-M36 Lead partner – UDUS Participants: BANGOR, UHAM, CSIC, CNR, IST-ID, FHNW, BSC, EUC
Task 4.6 Design of multi-enzyme blends to process complex ingredient mixtures M12-M40 Task Lead Partner – CSIC Participants: BANGOR, UDUS, UHAM, IST-ID

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D4.1	QR barcoding system, available	1 - CSIC	Other	Confidential, only for members of the consortium (including the Commission Services)	3
D4.2	The FuturEnzyme Portfolio of 1,000 enzyme (recombinant/ native/biomimetic) material, obtained	1 - CSIC	Other	Confidential, only for members of the consortium (including the Commission Services)	16
D4.3	Cell-free expression/ reported system, developed	4 - UHAM	Other	Confidential, only for members of the consortium (including the Commission Services)	16
D4.4	Biomimetic protease production system, developed	9 - FHNW	Other	Confidential, only for members of the consortium (including the Commission Services)	16
D4.5	At least 9 enzyme crystal structures	1 - CSIC	Other	Confidential, only for members of the consortium (including the Commission Services)	30
D4.6	The metadata on expression yield, activity and stability, available	5 - UDUS	data sets, microdata, etc	Confidential, only for members of the consortium (including the Commission Services)	18
D4.7	At least 180 enzymes (recombinant, native, biomimetic) with attractive properties, available	1 - CSIC	Other	Confidential, only for members of the consortium (including the Commission Services)	18
D4.8	Set of high-performing multi-enzyme blends	1 - CSIC	Other	Confidential, only for members of the consortium (including the Commission Services)	20

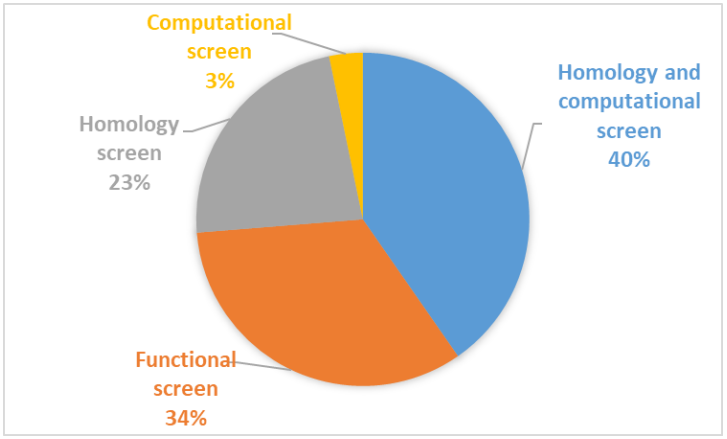
final version?

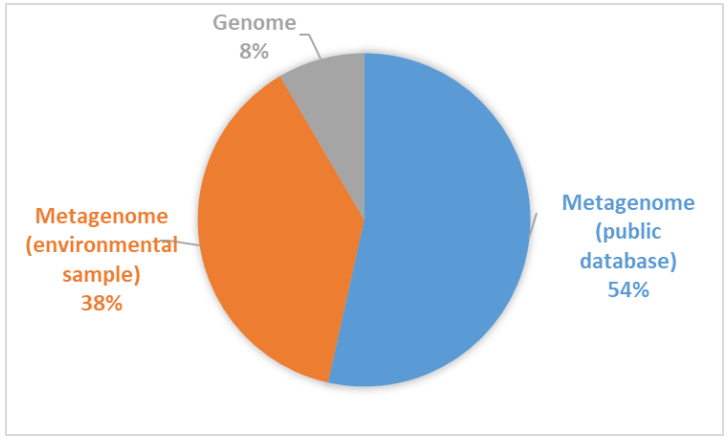
Task 4.1 Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production M2-M30

- Task Lead Partner – UHAM
- Participants: CSIC, BANGOR, UDUS, CNR, EUC

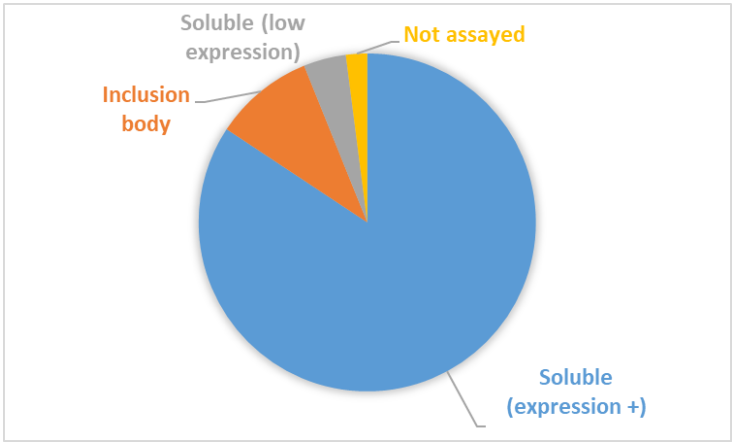
Task 4.1 Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production M2-M30

- Actually, CSIC has contributed to the cloning and synthesis of 344 genes
  - Lipases-esterases: 304
  - Hyaluronidases: 16
  - Polyester hydrolases: 10
  - Amidases: 6
  - Amylases: 4
  - PluriZymes: 3
  - Proteases: 1





- Expression in *Escherichia coli* (scale: 50 mL, 1 L, 4 L, … 12 L)
  - Ek/LIC 46
  - p15TV-L
  - pBXNH3
  - pET-45b(+)
  - pQE306



UDUS

Previosly reported

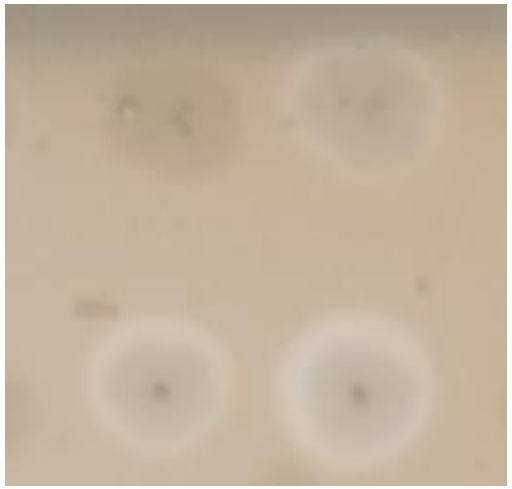
Focus: hydrolases [lipases, esterases, cutinases].

- WP4
  - Task 4.1 Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production

✓Esterase & lipase set: first tests for detergent compatibility (Henkel)

✓60 enzymes out of 85 lipolytic enzymes expressed in active form using *E. coli* in deep well plates for HTS with cell extracts

✓Cutinase-like enzymes productions studies in *B. subtilis* as alternative host to enhance soluble protein yields



B. subtilis producing the cutinase-like enzyme Paes_PEH from a signal peptide library on polyester indicator plates

WP4 Results obtained by Eucodis

Task 4.1. Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production (M2-30)

Goal: Improvement of current expression systems in WP4 to be able to produce enzymes in WP6 in the desired quantities for the downstream partners

Approach:

- **Expression in Pichia pastoris (ongoing, see results on next slides):**
  - Design of integration plasmids for faster cloning, establishment of secretion signal/pro-peptide library, fermentation optimization with improved plasmids, new promoters to be tested
- **Expression in Corynebacterium glutamicum (planned):**
  - Design of integration plasmids for stable integration into genome, Design and test of secretion signal peptide library, antibiotics-free expression for food/cosmetics grade enzymes

WP4 Results obtained by Eucodis

Task 4.1. Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production (M2-30)

Small scale screening of different Signal Peptides (SPs) using Cal B wildtype and Eucodis lipase 32 as model enzymes:

- Not predictable, which SP is preferred
- Screening necessary

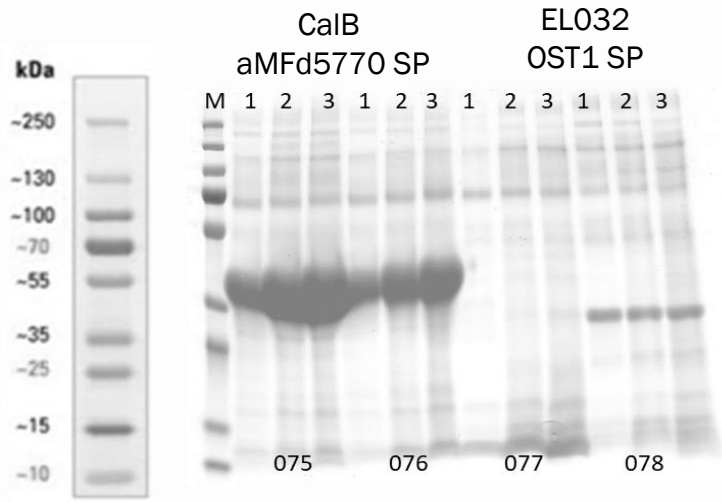
pos. ctrl:	A12/C12/E12/G12	CalB-KREAEA #8
neg. ctrl:	B12/D12/F12/H12	Pichia wt

	Units /ml	1	2	3	4	5	6	7	8	9	10	11	12
pPichia57ost1_CalBwt	A	0,331	0,385	0,373	0,355	0,355	0,350	0,367	0,358	0,365	0,351	0,374	0,340
pPichia57aMFd5770_CalBwt	B	0,447	0,393	0,393	0,409	0,402	0,363	0,419	0,422	0,413	0,431	0,424	0,008
pPichia57SPshort_CalBwt	C	0,196	0,109	0,128	0,122	0,204	0,121	0,133	0,147	0,111	0,146	0,170	0,290
pPichia57aMF_CalBwt	D	0,346	0,355	0,314	0,282	0,326	0,331	0,356	0,352	0,341	0,355	0,293	0,008
pPichia57ost1_ EL032	E	0,222	0,161	0,154	0,159	0,100	0,093	0,241	0,211	0,243	0,198	0,238	0,292
pPichia57aMFd5770_ EL032	F	0,147	0,155	0,089	0,065	0,107	0,058	0,061	0,067	0,058	0,082	0,053	0,010
pPichia57SPshort_ EL032	G	0,071	0,036	0,026	0,058	0,024	0,044	0,029	0,032	0,042	0,039	0,060	0,314
pPichia57aMF_ EL032	H	0,142	0,089	0,089	0,106	0,150	0,078	0,115	0,110	0,040	0,065	0,044	0,009

➢ Yields improved by 25-60%. Optimized Pichia Signal Peptides ready for Consortium enzyme production.

Secreted expression in Fermenters:

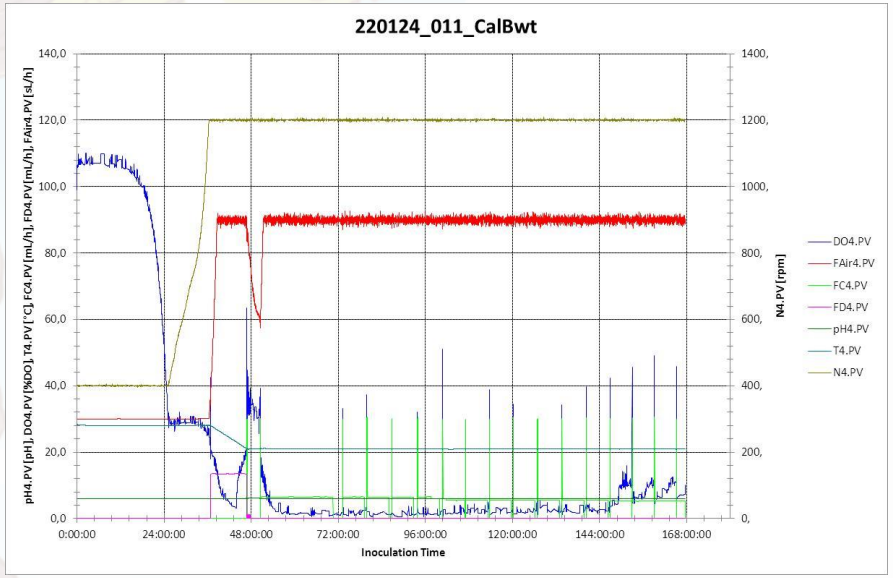
- High yields
- High purity



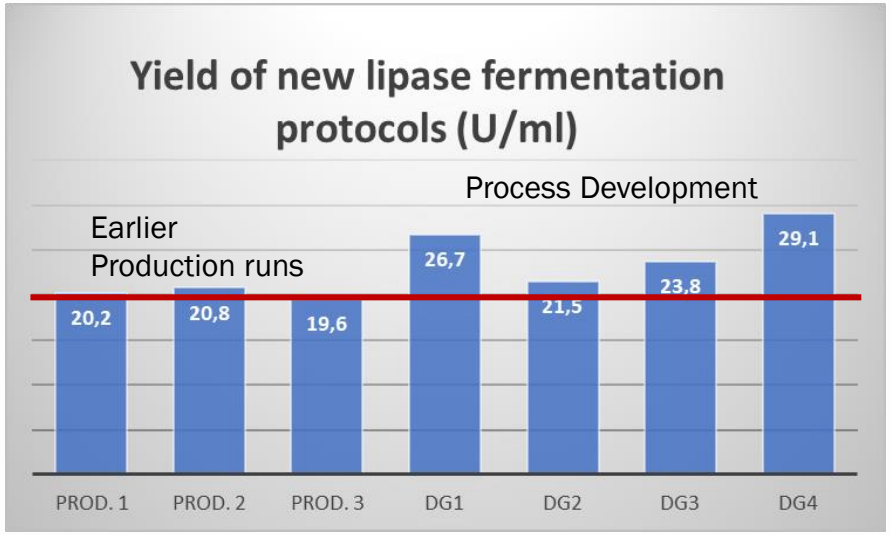
WP4 Results obtained by Eucodis

Task 4.1. Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production (M2-30)

- Improved Lipase fermentation protocols (using CalB wildtype as model enzyme)
  - Optimized feeding strategy (methanol/glycerol co-feeding)
  - Optimized induction strategy (spiked versus continuous, amounts, time)

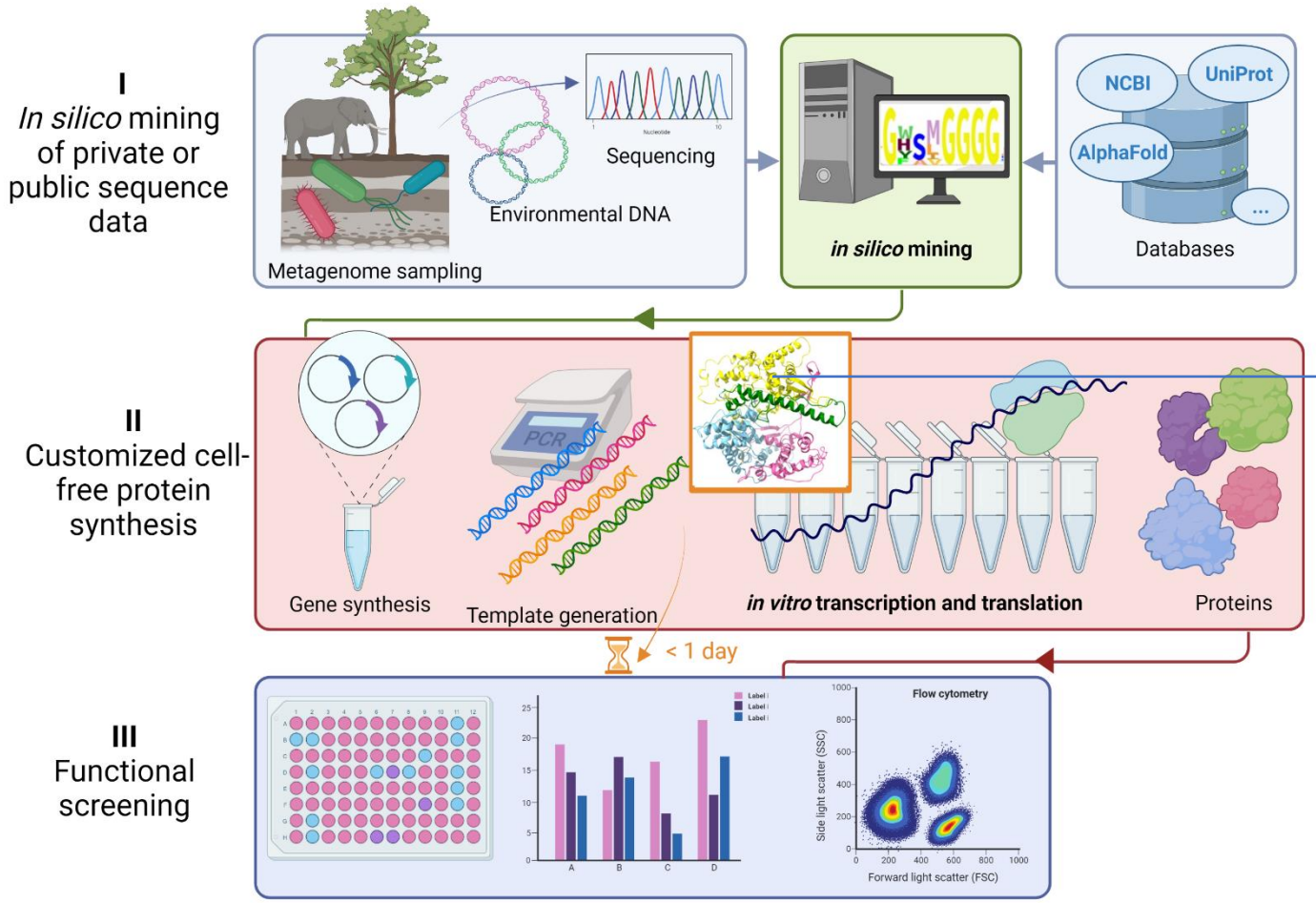


CalBwt as model enzyme



➢ Yields improved by 50-75%. Optimized Pichia protocols ready for Consortium enzyme production.

Task 4.1
Development of a cell-free expression system

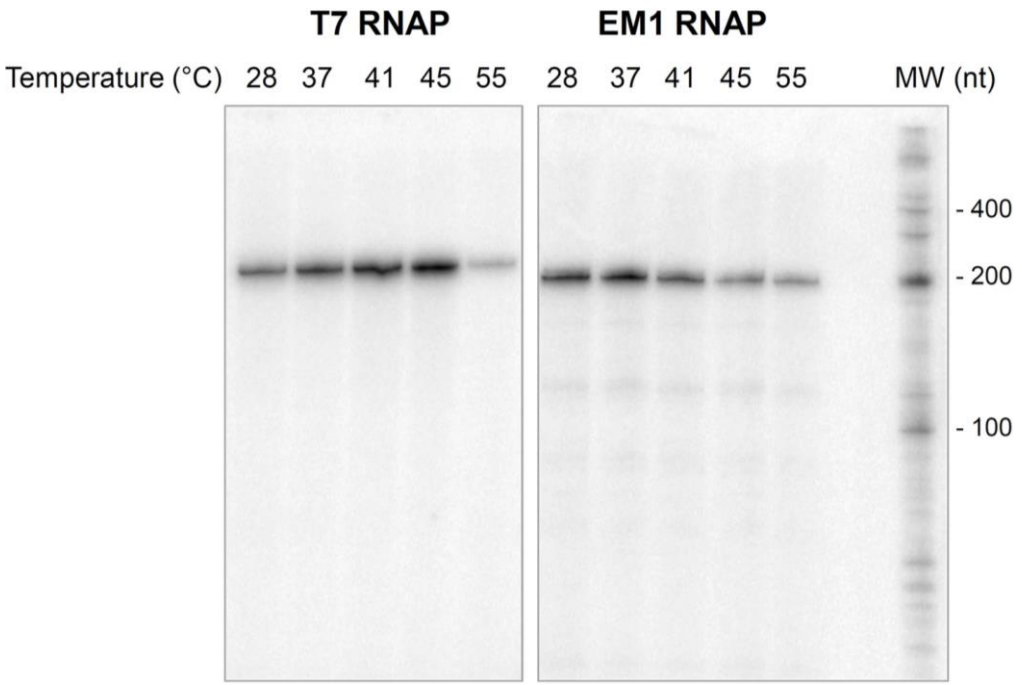


EM1-RNAP

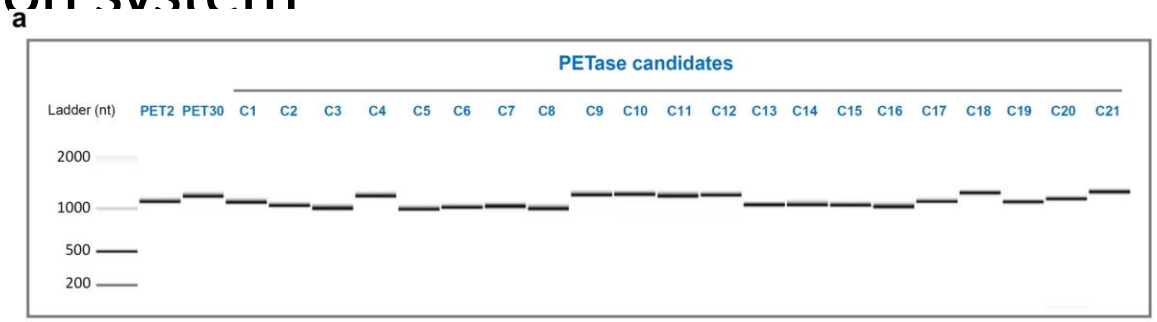
First viral RNAP from elefant faeces microbiome

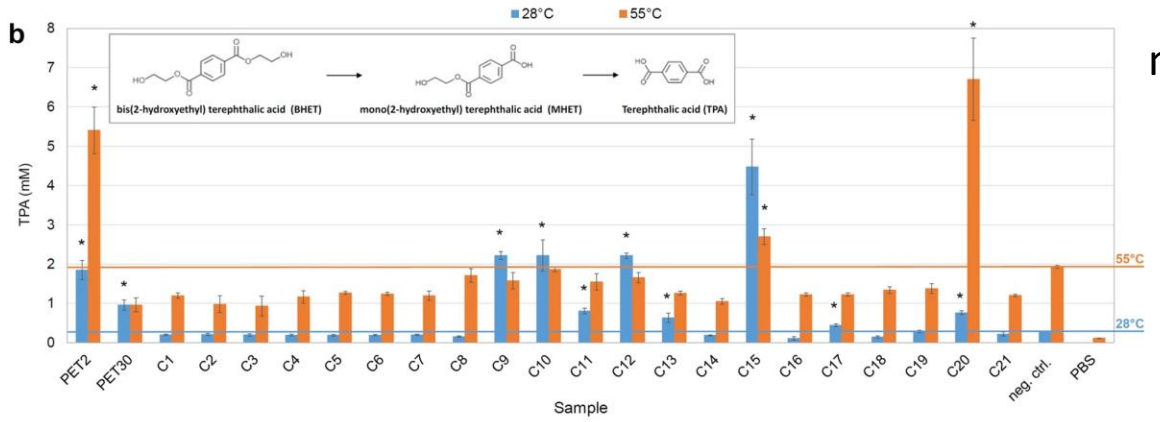
Han et. al. 2022, SciRep

Task 4.1
Development of a cell-free expression system

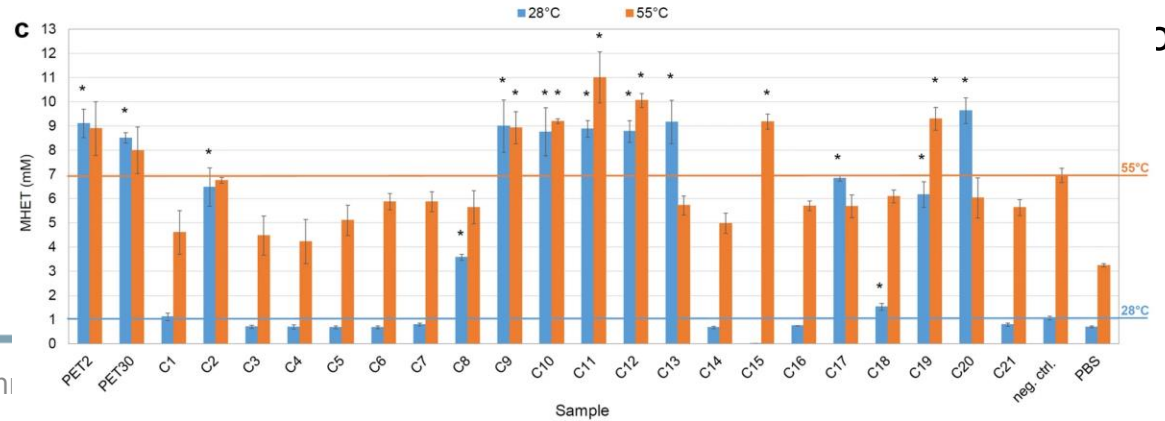


Activity equal to T7 RNAP
Applicability tested for PETases





m BHET



ɔm BHET

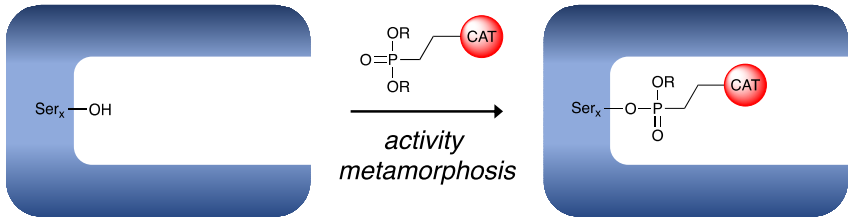
Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30

- Task Lead Partner: FHNW
- Participants: CSIC, EUC

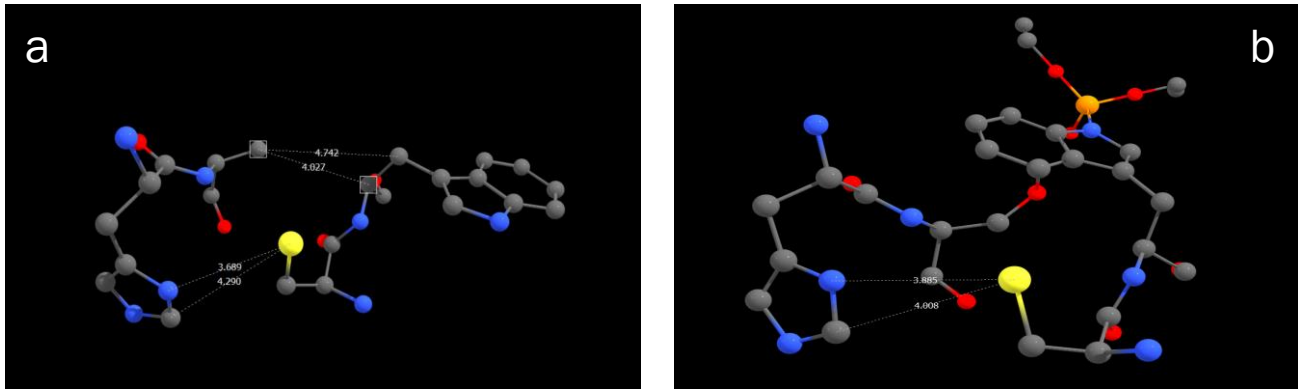
Ongoing activities

Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30

Design strategy



Artifical protease design - activity metamorphosis through the biocatalytic insertion of a synthetic catalyst in the active site of selected lipases (and other scaffolds). Currently are explored two classes of organocatalysts: serotonin derivative and short peptides; which need to bear phosphoester bonds (suicide inhibitors target for the lipase)

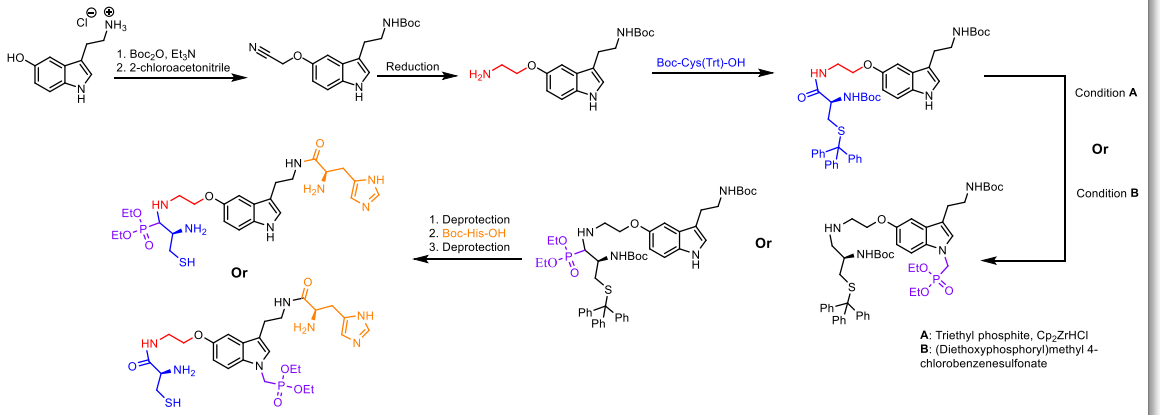


Papain : our model protease – Catalytic diad of papain (left) where Cys25 sulfhydryl function is activated by His159 (distance N-S = 3.7 Å) and catalytic inhibitor proposed (synthesis ongoing) where His and Cys (expected distance N-S = 3.9 Å) residues are attached through a semi-rigid linker (prolyl-indole).

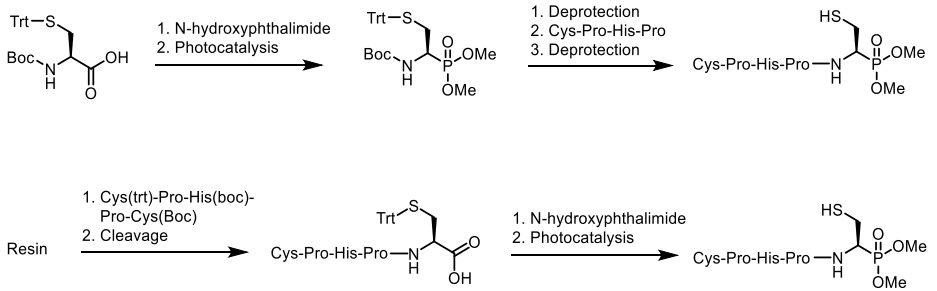
Ongoing activities

Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30

Synthetic work



Catalytic inhibitor synthesis (serotonin derivatives) – Initially, serotonin is protected by a *t*-butyloxycarbonyl (Boc) group to allow for a regioselective Williamson etherification of the free hydroxyl group followed by the reduction of the cyano moiety introduced to yield the corresponding primary amine. This amine function serves as anchoring point to attach a protected Cys residue. Two phosphorylation pathways are tested along with selective deprotection reactions to yield a catalytic suicide inhibitor.



Catalytic inhibitor synthesis (peptide derivatives) – A first peptide sequence has been designed and the synthetic challenge lies in the introduction of phosphonate groups at the C-terminus of the peptide. This is carried out using a method recently published based on visible light photocatalysis (*Angew. Chem. Int. Ed.* **2022**, *61*, e202207063). Peptide synthesis is carried out with a newly acquired automatic peptide synthesizer using appropriate protecting groups.

Ongoing activities

Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30

Beyond lipases: *de novo* protein scaffolds to design artificial proteases

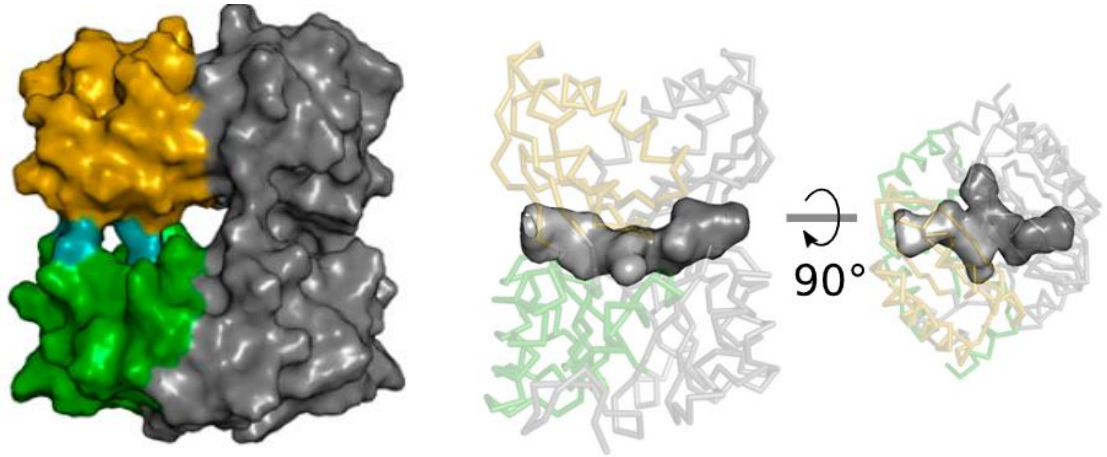
de novo protein selection & production

↓ *In silico introduction of esterase catalytic triad (BSC)*

Artificial Lipase

↓ *Metamorphosis (with catalytic suicide inhibitor)*

Artificial Protease (and more)



Selected *de novo* protein – the selected scaffold displays a large cavity can serve as binding pockets and/or enzymatic reaction chambers. It also display high stability (95 °C, guanidinium chloride 2M) suggesting it should tolerate considerable modification of the residues surrounding the cavities.

Ongoing activities

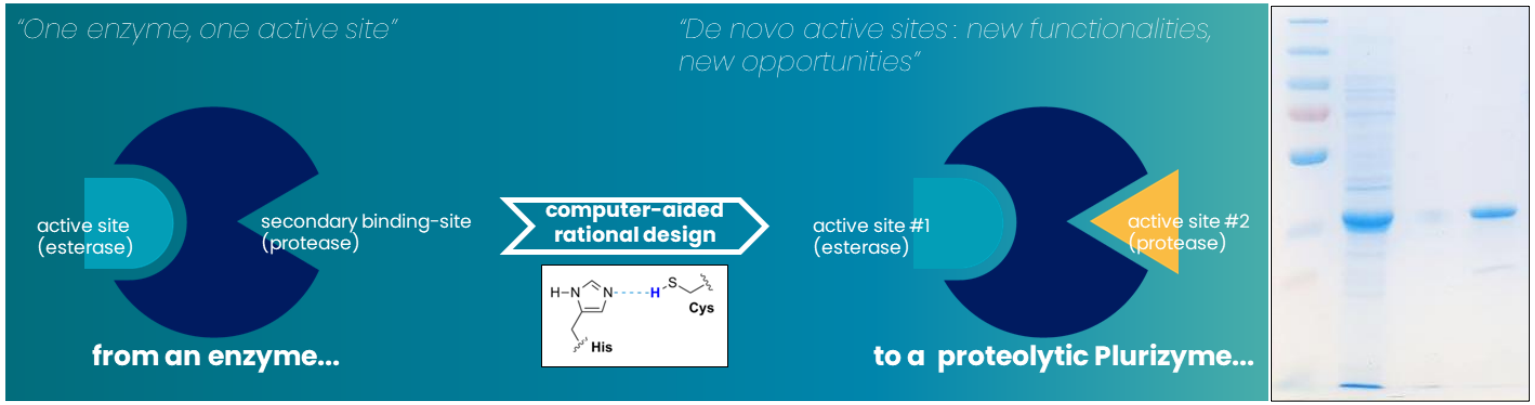
Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30

Work status

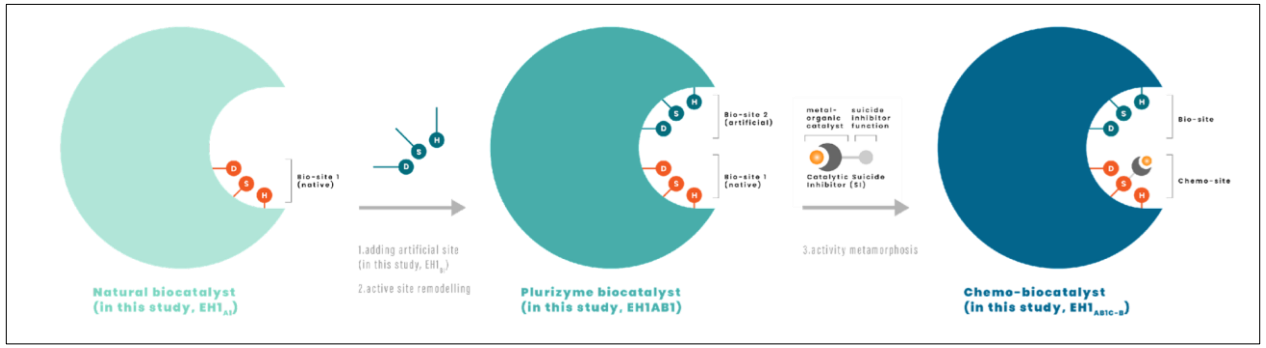
- Synthetic inhibitor synthesis is well advanced, and first inhibitors are expected to be available by end of Q4 2022.
- Peptide synthesis is ongoing. Once the phosphorylation reaction is established, a large number of different peptides can be produced.
- All inhibitors produced will be tested for their ability to inhibit *FuturEnzyme* lipases and, further, for their proteolytic activity (model substrates, casein)
- BSC, FHNW and CSIC are working to engineer and endow the selected *de novo* protein with ester hydrolase activity. Different mutants will be produced by FHNW and tested for esterase activity. All inhibitors produced will be tested with the newly engineered esterase.

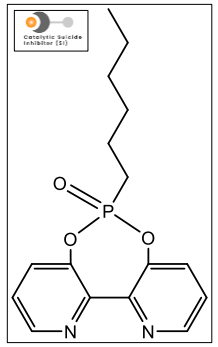
Task 4.2 Smart design systems to obtain enzymes with inherent problems of expression M2-M30

- CSIC, BSC, EUCODIS, FHMW has contributed to the production of two novel expression systems
  - *PluriZyme* system, for producing artificial proteases



  - *Biomimetic* system, for producing biomimetic oxidases





CSIC BSC EUCODIS BIOSCIENCE

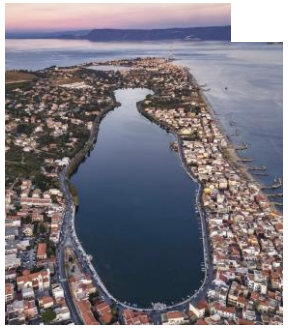
n|w University of Applied Sciences and Arts Northwestern Switzerland

Task 4.3 Production of enzymes from their natural hosts M2-M30

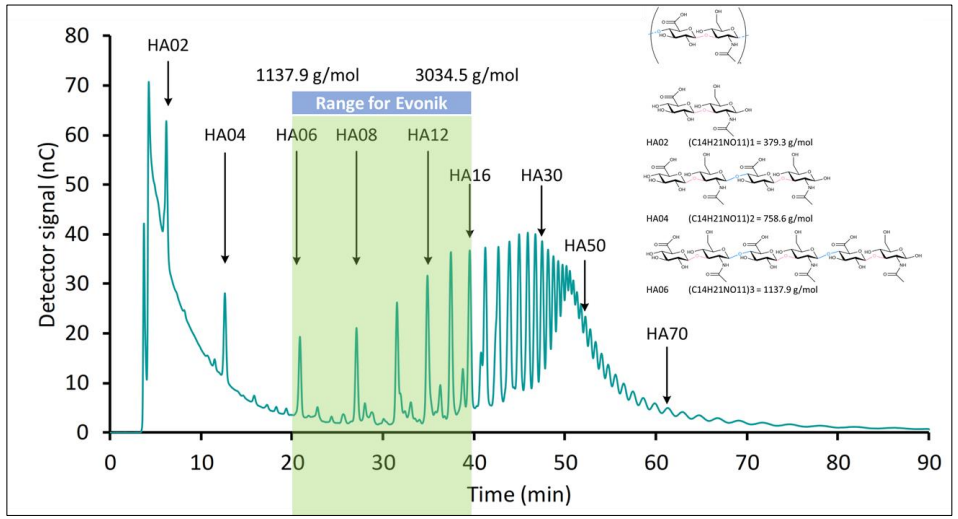
- Task Lead Partner – CNR
- Participants: IST-ID, ACTY

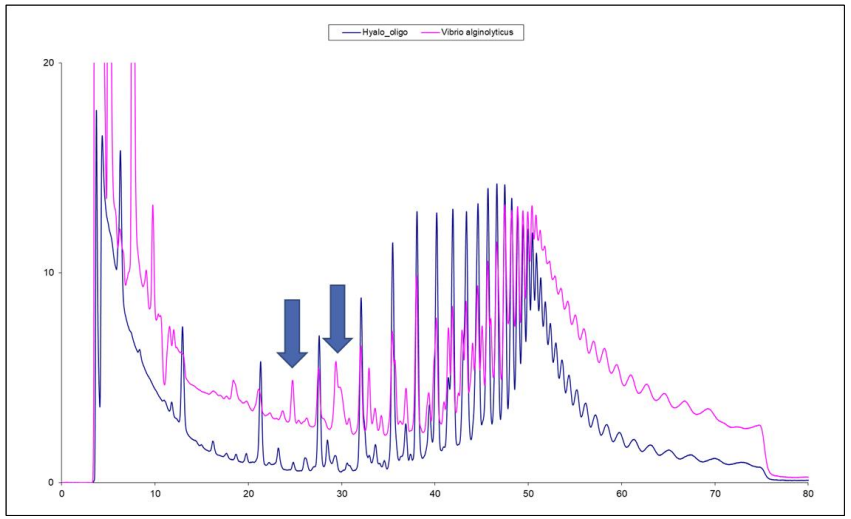
Task 4.3 Production of enzymes from their natural hosts M2-M30

- CNR: *Vibrio alginolyticus*, the best isolate producing hyaluronidases
  - *Two strains selected*
    - *Vibrio alginolyticus_V4*
      Basiluzzo submarine hydrothermal field, Panarea Island, Aeolian Archipelago, South Tyrrhenian Sea (38°40.315′N; 15°07.846′E)
    - *Vibrio alginolyticus_#23 Lake of Ganzirri, Sicily, Italy (38°15′39.95″N 15°37′01.9″E)*



CULTIVATION CONDITION: Artificial seawater
Medium supplemented with 0.2% HA



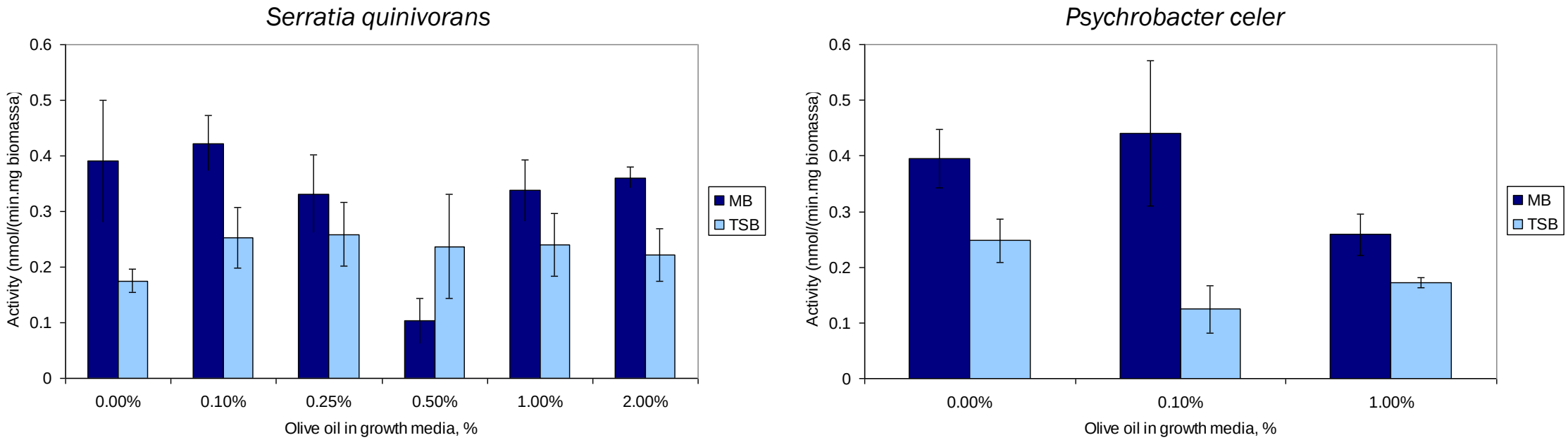


Task 4.3 Production of enzymes from their natural hosts

- conditions for cultivation of microbes for highest enzyme activities; assessment of enzyme performance under industrial relevant conditions; testing enzymes in small scale bioreactors

Lipases/Esterases

Effect of media composition and olive oil concentration during growth on strain activity



MB = marine broth; TSB – tryptic soy broth

Task 4.4: Enzyme characterisation for selecting those with manufacturers' specifications M2-M36

- Task Lead Partner – UDUS
- Participants: BANGOR, CSIC, UHAM, FHNW, IST-ID, EUC

UDUS

Previosly reported

- WP4
  - ➢ Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

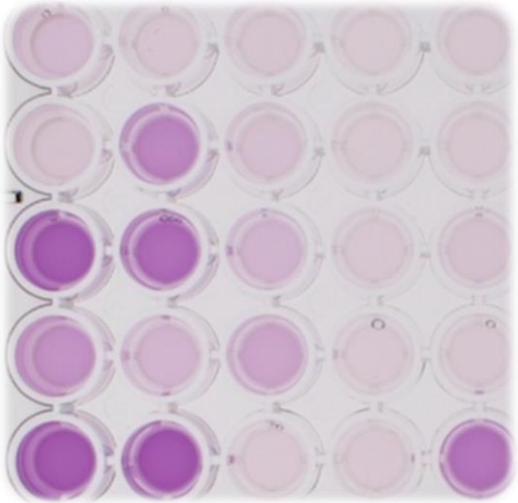
Application: Detergents

✓ Activity screening of esterase/lipase collection with industry-oriented substrates using the NEFA kit

➢ Detection of **N**on-**E**sterified **F**atty **A**cid (NEFA) in a two-step colorimetric assay

➢ Substrates: standardized stains of:

1) beef fat on polyester/cotton mix
2) lipstick on polyester/cotton mix
3) collar stain on polyester/cotton mix



UDUS

- WP4
  - ➢ Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

Application: Detergents

✓ Activity screening of esterase/lipase collection with industry-oriented substrates using the NEFA kit

Enzyme	Detergent stability		Detergent stability
LCC WCCG	**yes**	LCC	**yes**
PETase	no	Pbau PE-H	**yes**
Ppel PE-H	n.d.	POIL-1 PE-H	n.d.
Psab PE-H	no	Poce PE-H	**yes**
Hyd18c13	**yes**	Paes PE-H Y250S	**yes**
TBEc350	no	Paes PE-H (CE16)	**yes**
Paby PE-H	n.d.	Pxin PE-H	**yes**
Abo_LipA (CE02)	no	TBEc304	**yes**
TBEc310	**yes**	CycTB025	**yes**
Abo_LipD (CE07)	no	Dim-008 (CE01)	no
Abo_Est3 (CE03)	no	Est24c11	n.d.
		Hyd8c31	no
		1,4-D#003	no
		Est24c4	**yes**

stain	number
beef fat	6
lipstick	5
both	14
in total	25

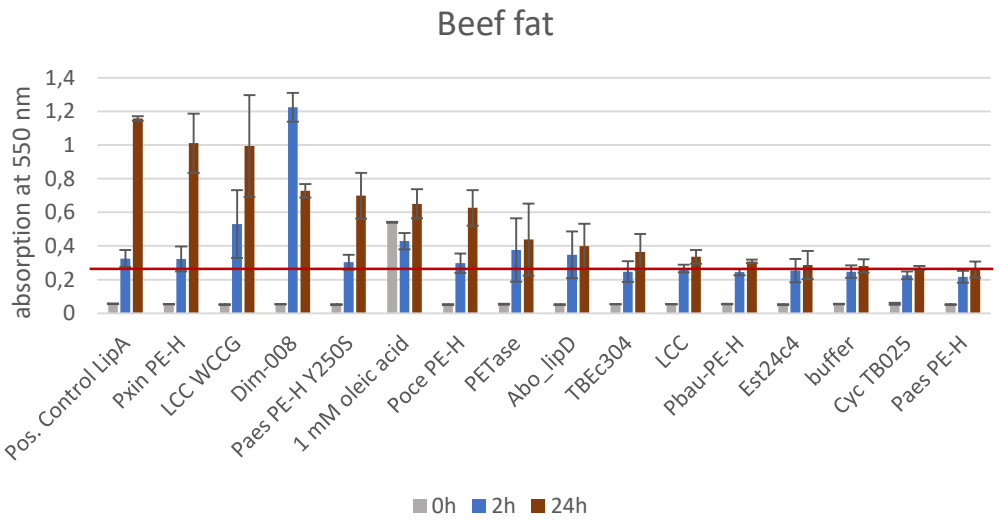
- ➢ 25 enzymes with activity on beef fat, lipstick or on both stains
- ➢ Many of them are detergent-stable
- ➢ Next step: purification and re-evaluation

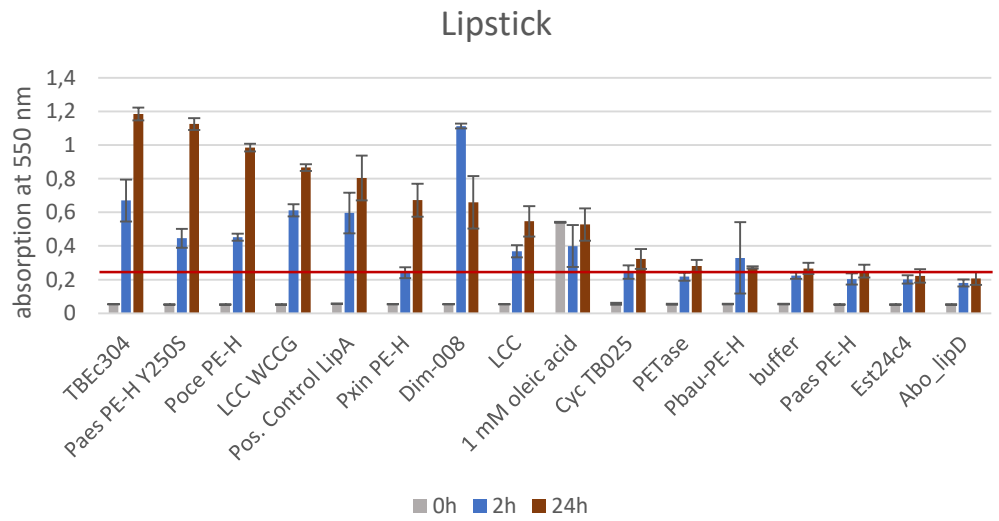
UDUS

- WP4
  - ➢ Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

✓ Activity screening of esterase/lipase collection with industry-oriented substrates using the NEFA kit

Application: Detergents





- ➢ 13 enzymes could be purified, 11 showed activity on beef fat and 10 on lipstick stains
- ➢ 6 of those enzymes offer elevated detergent stability

UDUS

- WP4
  - ➢ Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

✓ Confirming hyaluronic acid degradation in culture supernatant of the isolates

Application: Cosmetics

Cultivation of the isolates with HMW-Hyaluronic acid (2MDa, Sigma-Aldrich) analysis of supernatants with 1% agarose gel, dying: Stains-All (0.005% in 50% EtOH)

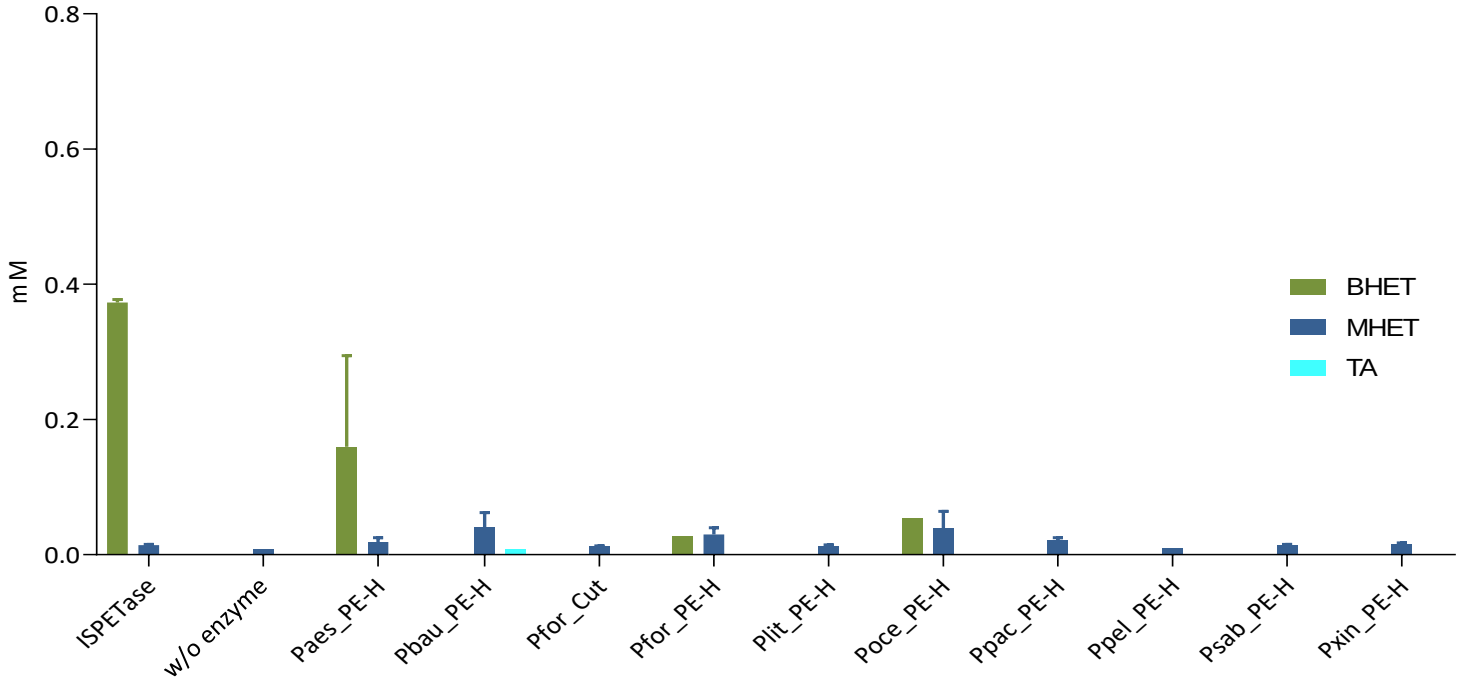
UDUS

- WP4
  - ➢ Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

Application: Textiles

✓ PET Monomer release from Schoeller sample textile

➢ Substrate: sample textile 4-b 3X58 (VORB, 100% PES 100g/m^2) pretreated by alkaline boiling



1 mg/ml enzyme; 168 h incubation, 30°C; 500µL reaction mix

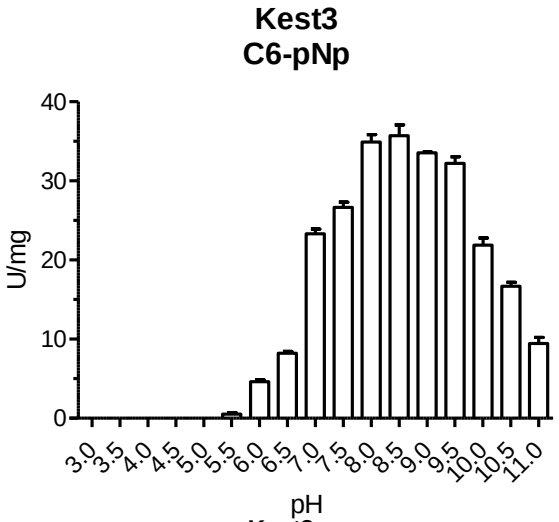
ENZYMES FOR INDUSTRIAL APPLICATIONS:

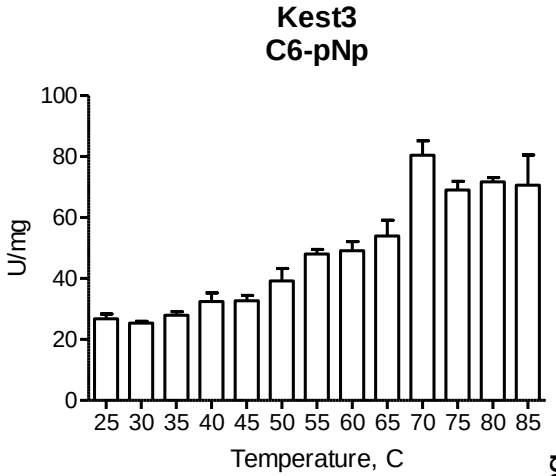
AMYLASES/LIPASES/PETases/CELLULASES/ HYALURONIDASES

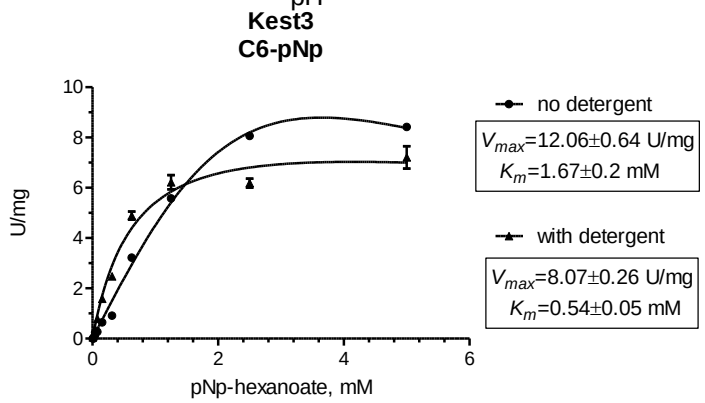
- BU collection of 39 amylases were expressed.16 were purified soluble, screened with 20 natural substrates
- ★ BU collection of 56 esterases :47 enzymes purified soluble, 21 active with C18 esters were identified and selected for activity screen with olive, palm and coconut oils
- ★ 47 soluble esterases were screened with 3PET, PCL and PLA plastic suspension
- ★ BU collection: 23 cellulases were expressed, 12 purified soluble and screened against 20 natural substrates
- 8 hyaluronidases from metagenomes were cloned, expressed. 6 were purified soluble and screened against hyaluronic acid different chain length

Thermostable lipases

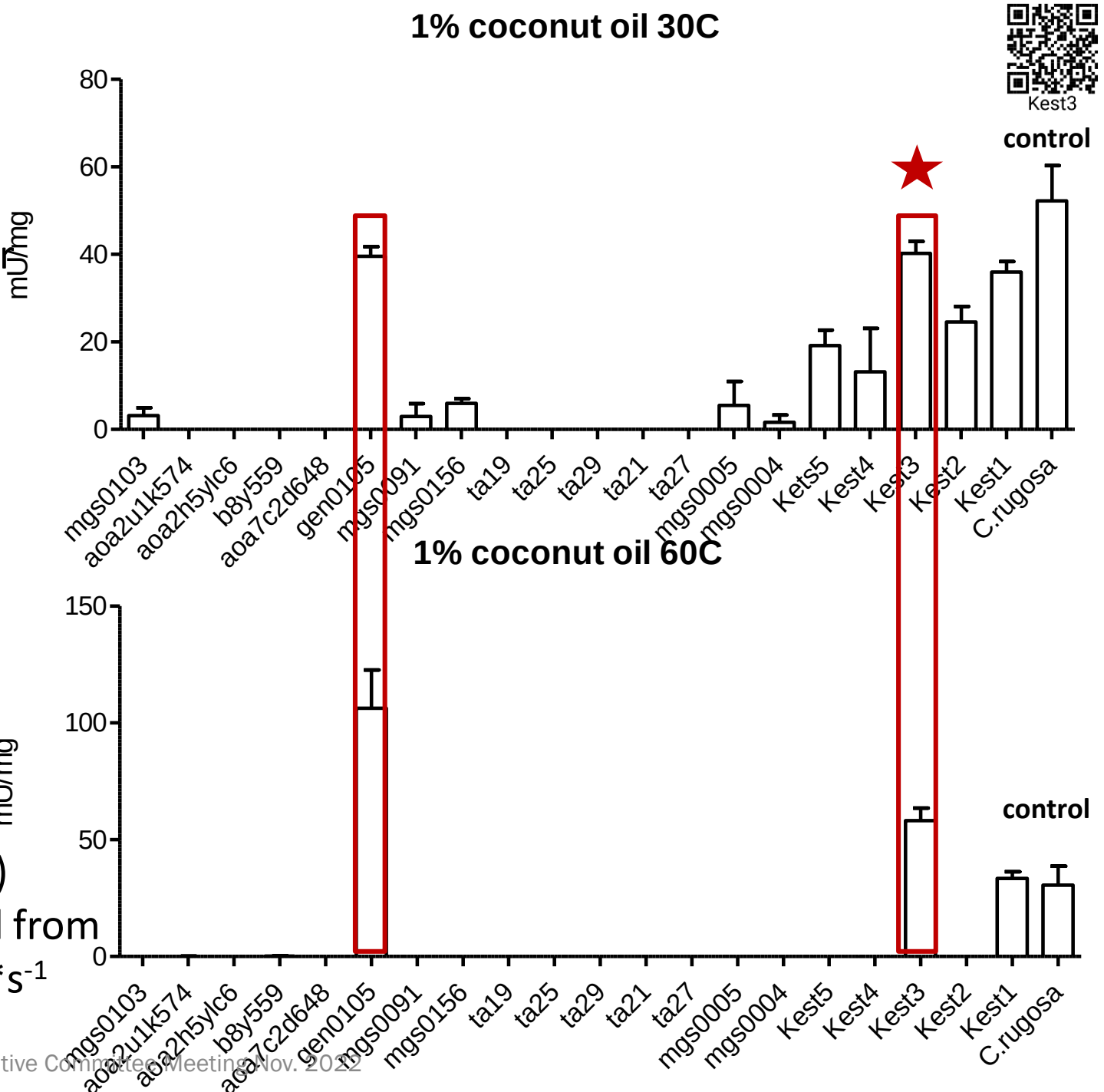
1 good candidate selected by activity and thermostability: KEST3. Sequence originates from *Fervidobacterium riparium*





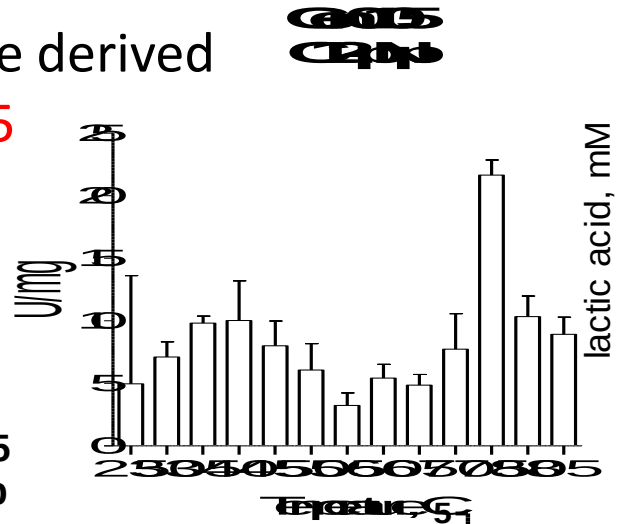


In presence of detergent (2.5%) k_{cat}/K_m **increased** from 4.1 to 9.3 $mM^{-1}*s^{-1}$

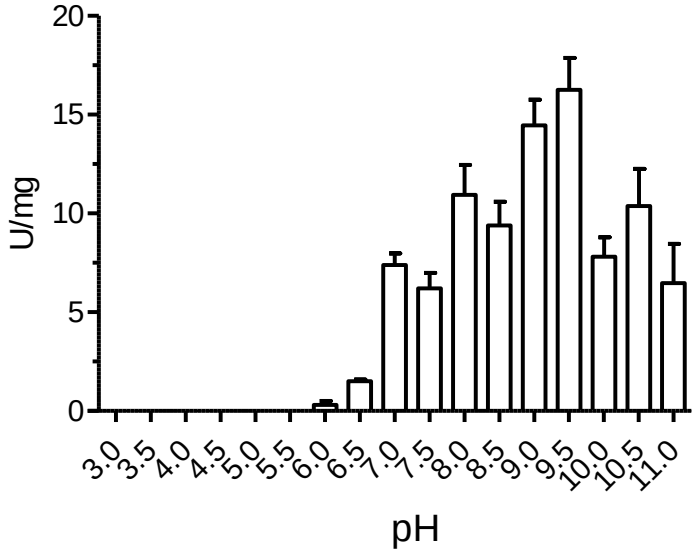


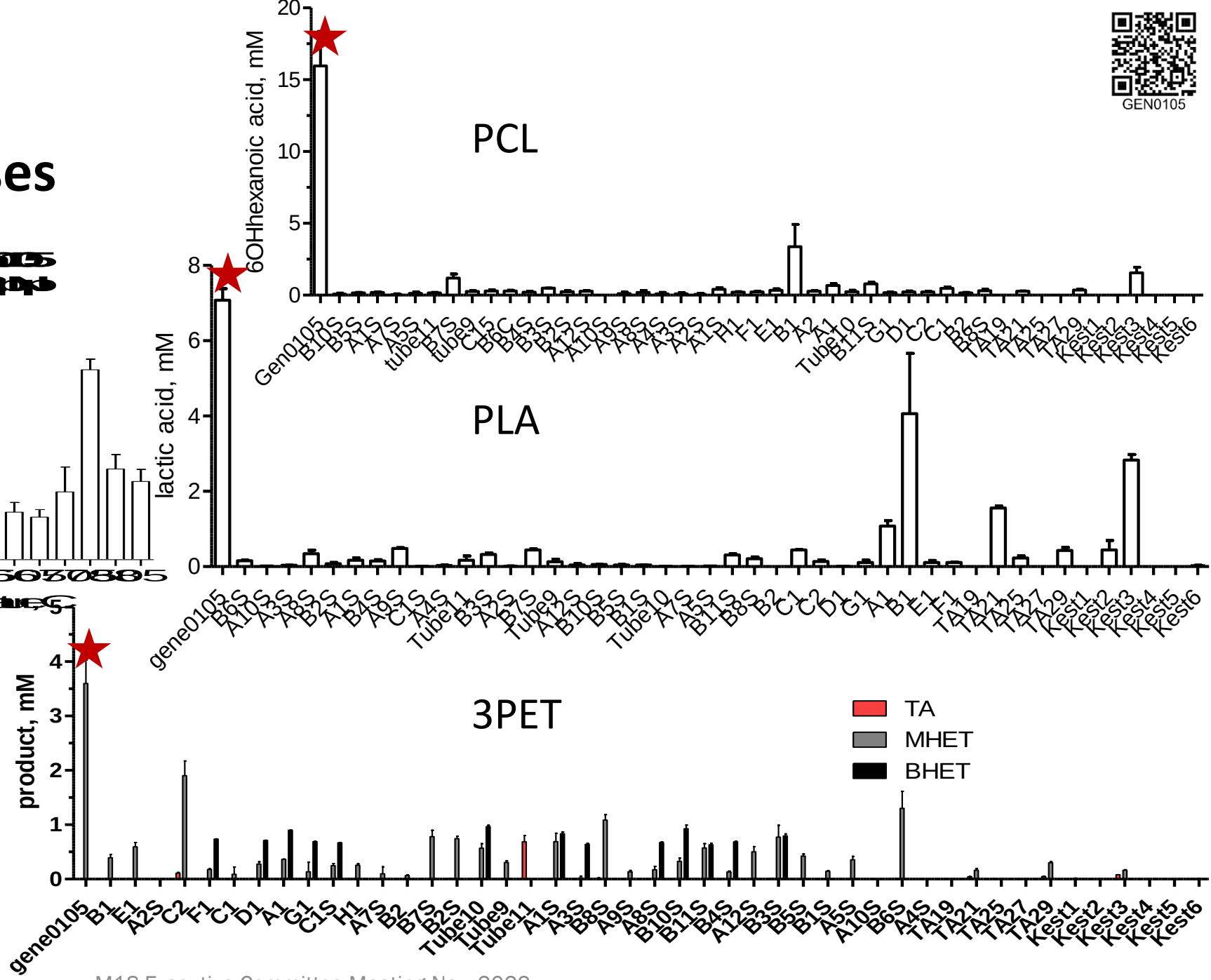
Plastic active esterases

One metagenome derived esterase GEN0105 was selected



Gen0105
C12-pNp

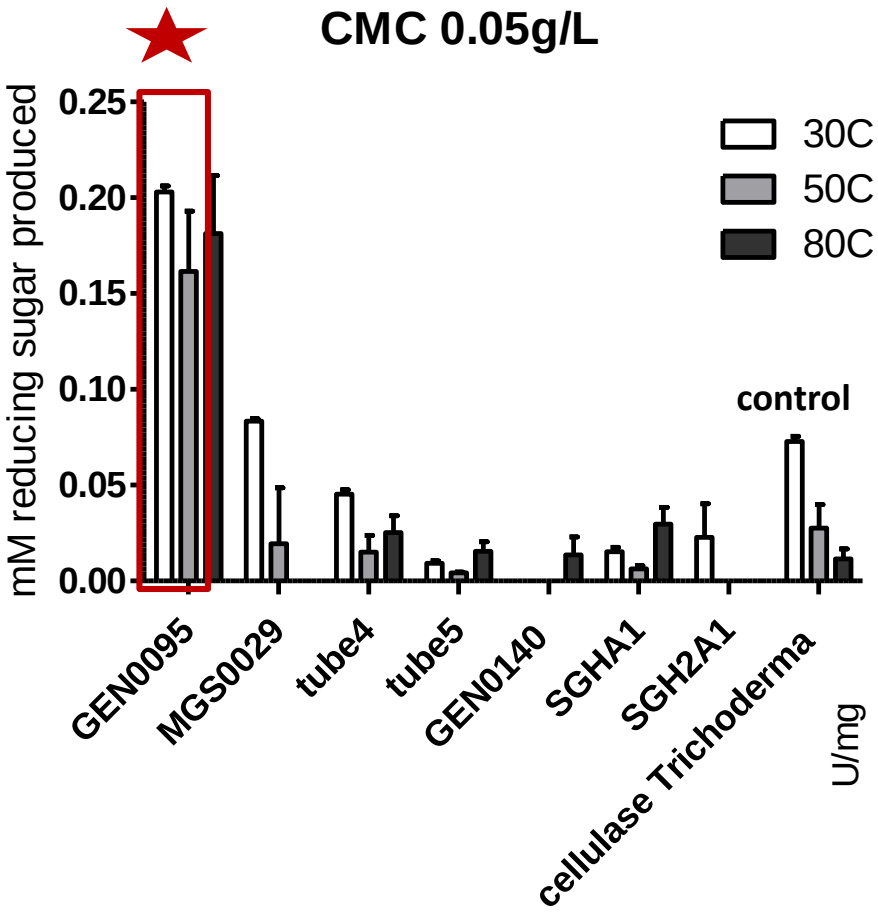




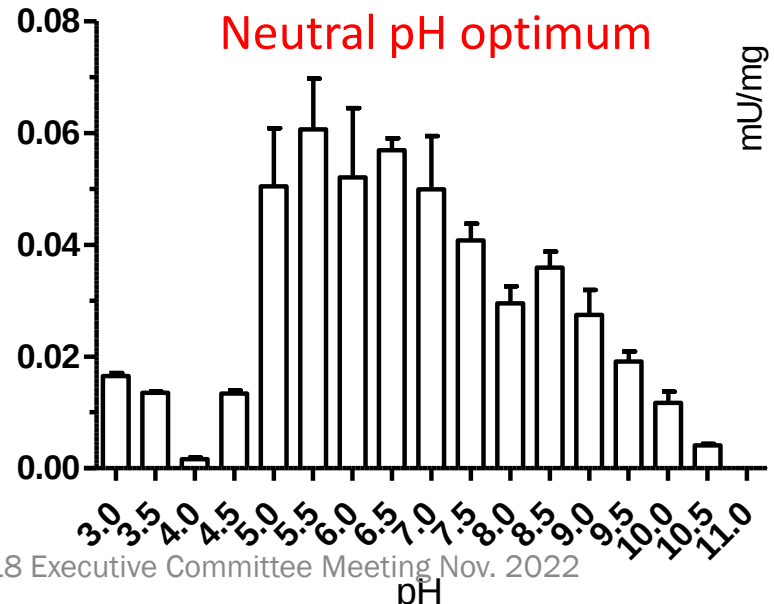
GEN0105

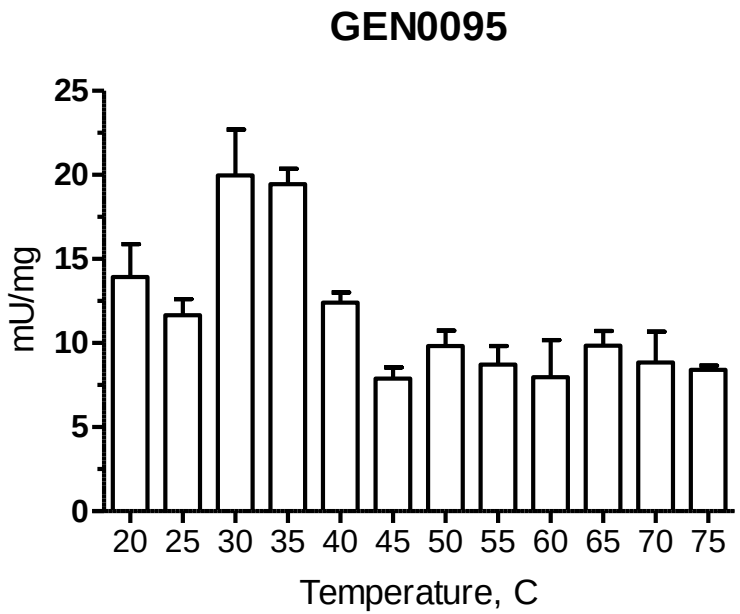
gen0095

Thermostable cellulases



12 cellulase screened using BCA and DNS assay 7 active candidates were screened at 30, 50 and 80C for activity with CMC. One active candidate GEN0095 from metagenome screen was selected.





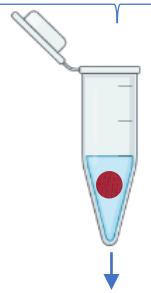
Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications M2-M36

- Extensive characterization: two lipases selected for the detergents and textile applications
  - Lip9
    - Produced in E. coli, pET-45b(+) vector
    - Sequence:
      MAHHHHHHVGTGSNDDDDKSPDPMAEHNPVVMVHGIGGASYNFFSIKSYLATQGWDRNQLYAIDFIDKTGNNRNNGPRLSRFVKDVLDKTGAKK
      VDIVAHSMGGANTLYYIKNLDGGDKIENVVTIGGANGLVSSRALPGTDPNQKILYTSVYSSADLIVVNSLSRLIGARNVLIHGVGHIGLLTSSQVKGYIKEG
      LNGGGQNTN
    - Origin: Marine Metagenomics Database (MarRef)
    - Properties: Td 41,7ºC; Topt 30ºC; pHopt 9.0; stable in washing liquor (days), production 1 mg per L
  - ID9
    - Produced in E. coli, pET-45b(+) vector
    - Sequence:
      MAHHHHHHVGTGSNDDDDKSPDPMTNLSKPIPNPREYPILPPDMNYIYFENAHLFPFEPEKRDYSPVNAWWLSECAFLVYCHPGFARMAMALVGF
      DHFHFFQGKGTECMVSWNKDSIIVAFRGTEMKSLSAFHELRTDLNTAPVDFDKGSKVHKGFLKGLQEIWEGEEGLKLFLETLSAEAPSRSMWICGHSL
      GGALAALCFARLEKASGLYIYGAPRIGDGEFVRICDNRPVWRVEHGRDPIPLVPPDVPALNFNFKDMGKLIYIDYRGEILFERPLVTVEEEKSKVLLNISQQ
      RKRRESLSVEGFKGVLDKDRAKTLINGINEHIMQSRVEWKEYFDSLDKGIGLKIKDHMPIYYCAKLWNILIEGL
    - Origin: Metagenome from marine bone-degrading microbiome
    - Properties: Td 45.5ºC; Topt 40ºC; pHopt 9.5; stable in washing liquor (> weeks), production 63 mg per L

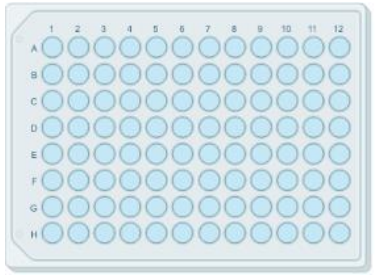
Pigment with oil on polyester/cotton PC-09	Mayonnaise on cotton C-S-05S	Lipstick, pink on polyester/cotton P-S-16	Fluid make up on cotton C-S-17	High discriminative sebum BEY on polyester/cotton PC-S-132	Beef fat on cotton C-S-61	Butterfat on cotton C-S-10

CSIC

100 µl Washing liquor (2.5 g/L in water)
24 hours
30°C
950 rpm

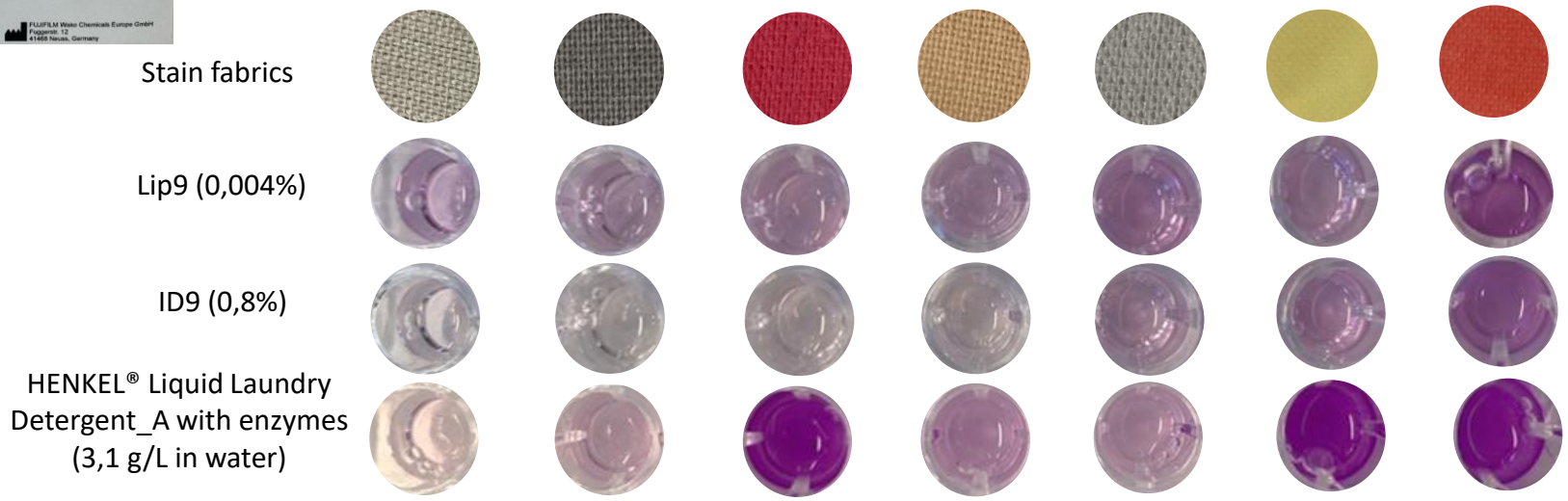


Lipase Lip9 at 0.004% w/v
Lipase ID9 at 0.8% w/v
HENKEL® Liquid Laundry Detergent_A with enzymes (3,1 g/L in water)



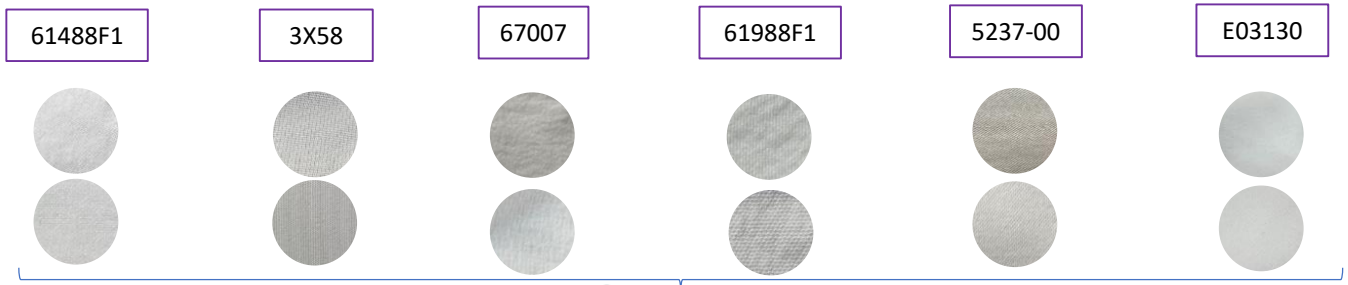
NEFA –Kit of Wako

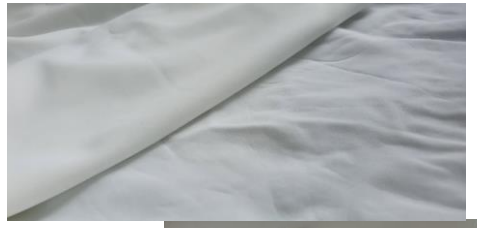
1. 10 µl "wash test" + 100 µl of R1a → 6 min, 37 °C
2. Add 50 µl of R2a → 6 min, 30 °C
3. Measure absorbance at 550 nm



Conclusions:

- Lipase ID9 shows activity towards butterfat (C-S-10) stain and with beef fat (C-S-61) stain, possibly because they have a similar fatty acid composition.
- Lip9 shows activity towards all the stains and specially high activity versus butterfat (C-S-10).
- HENKEL® Liquid Laundry Detergent_A including enzymes also shows activity with all of the stained cloths but it shows specially high activity towards lipstick (P-S-16), beef fat (C-S-61) and butterfat (C-S-10) stains.

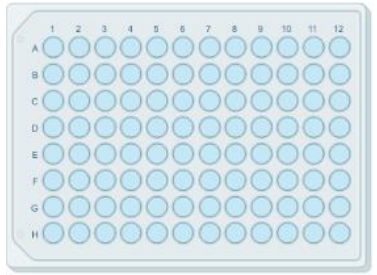




100 µl HEPES Buffer

24 hours
30°C
950 rpm

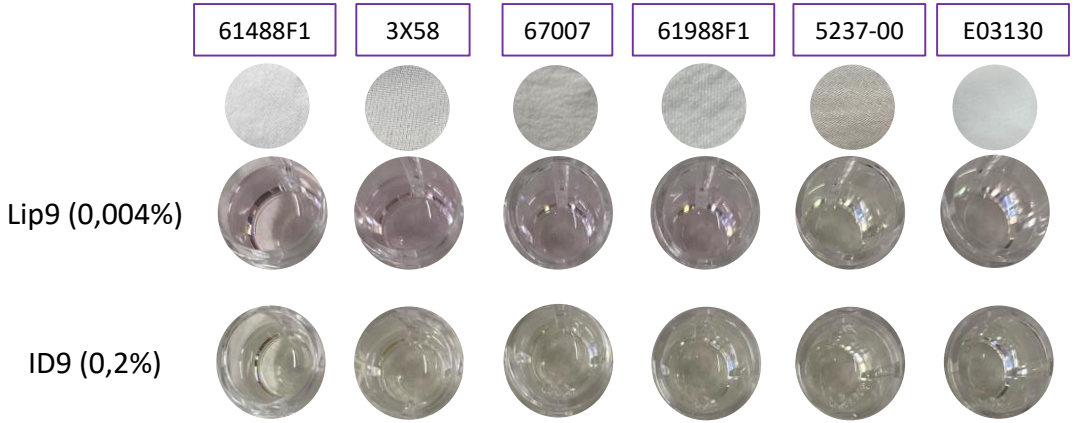
Lipase Lip9 at 0.004% w/v
Lipase ID9 at 0.2% w/v



NEFA –Kit of Wako

1. 10 µl "wash test" + 100 µl of R1a → 6 min, 37 °C
2. Add 50 µl of R2a → 6 min, 30 °C
3. Measure absorbance at 550 nm

Raw fabric



Conclusions:

- Lipase ID9 shows no capacity to clean the oils in the fabrics.
- Lipase Lip9 shows a broad capacity to clean the oils in all raw textiles provided by Schoeller. However, it is surprising that "cleaning" (by meanign of free fatty acid release) is detected in the raw and pretreated materials, the last one expected to contain low amount of chemicals or no chemicals.
- The next step will be to perform the "cleaning tests" in water, as requested recently.

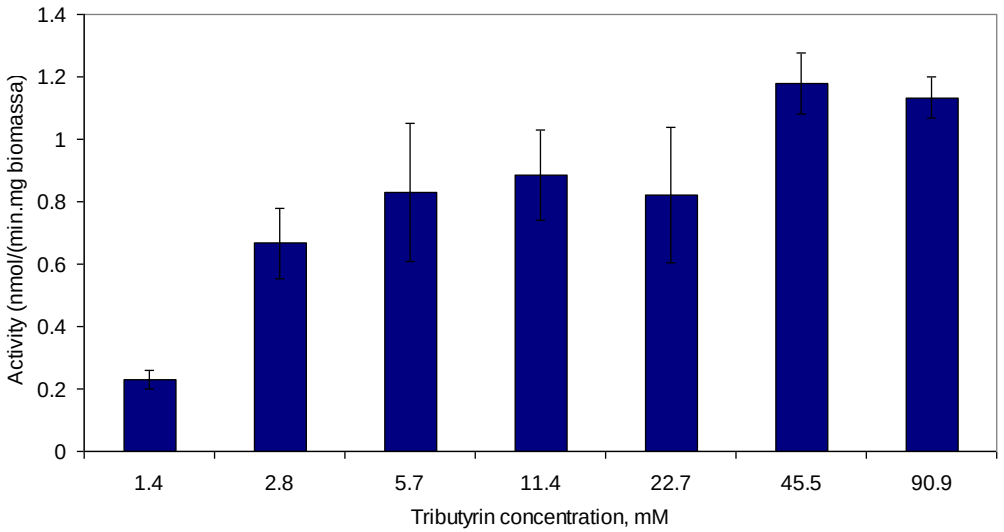
Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

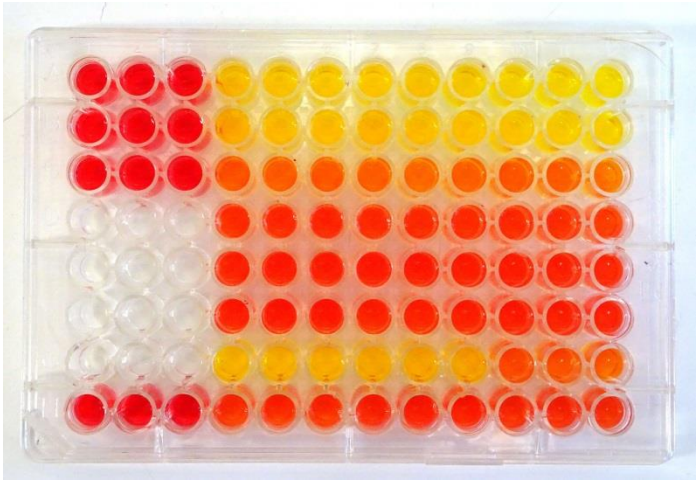
- conditions for cultivation of microbes for highest enzyme activities; assessment of enzyme performance under industrial relevant conditions; testing enzymes in small scale bioreactors

Lipases/Esterases

Effect of substrate concentration

Serratia quinivorans



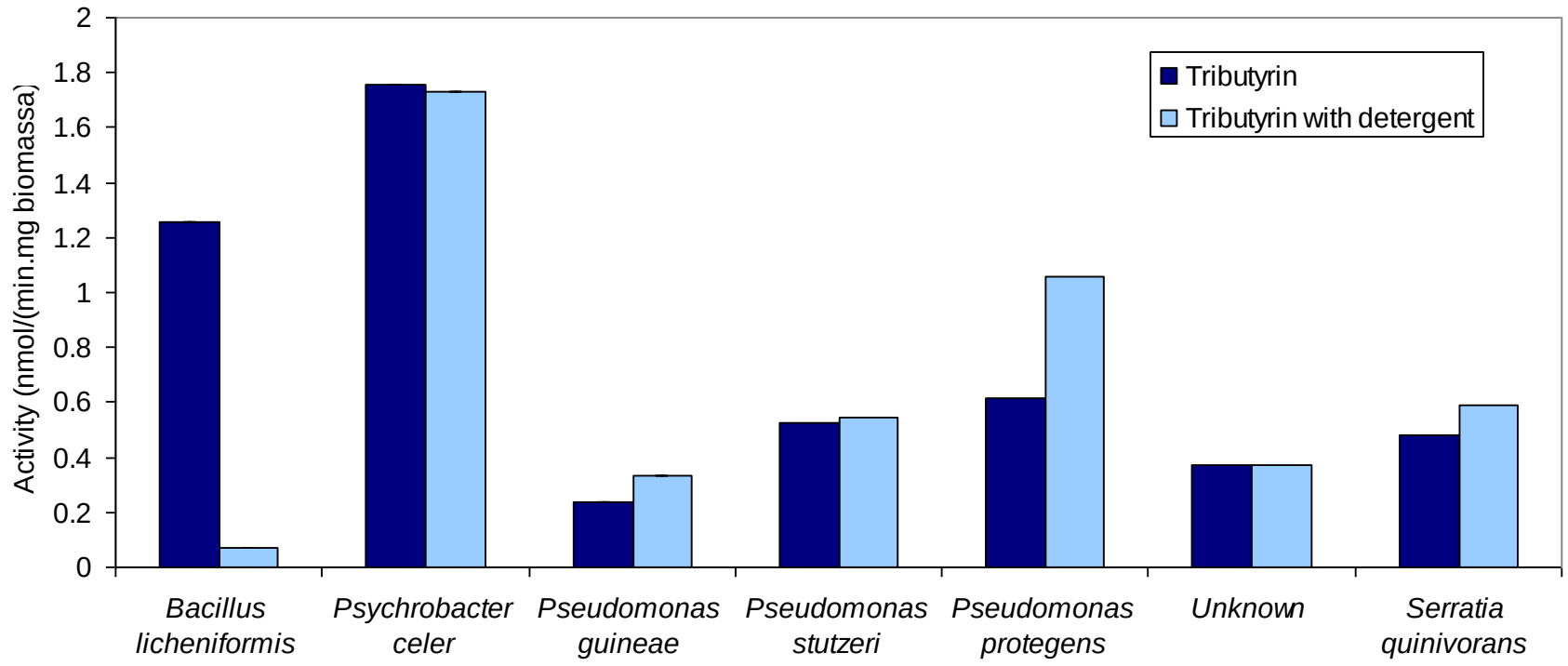


Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

- conditions for cultivation of microbes for highest enzyme activities; assessment of enzyme performance under industrial relevant conditions; testing enzymes in small scale bioreactors

Lipases/Esterases

Effect of detergent

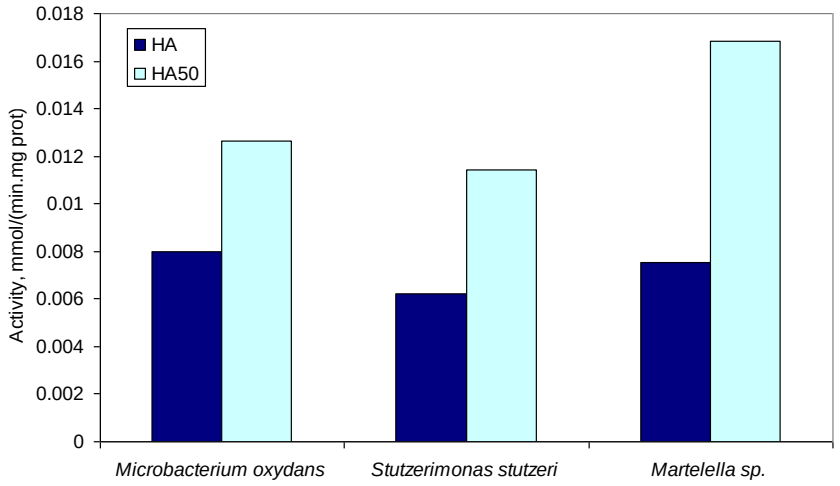


Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

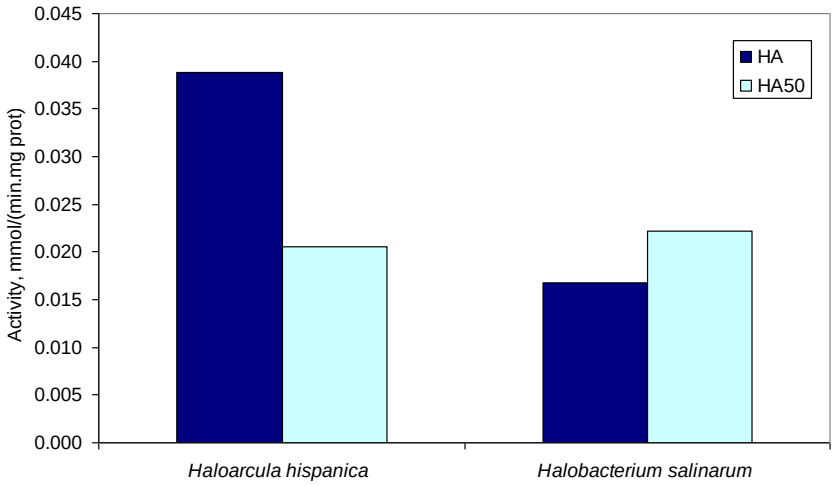
- conditions for cultivation of microbes for highest enzyme activities; assessment of enzyme performance under industrial relevant conditions; testing enzymes in small scale bioreactors

Hyaluronidases

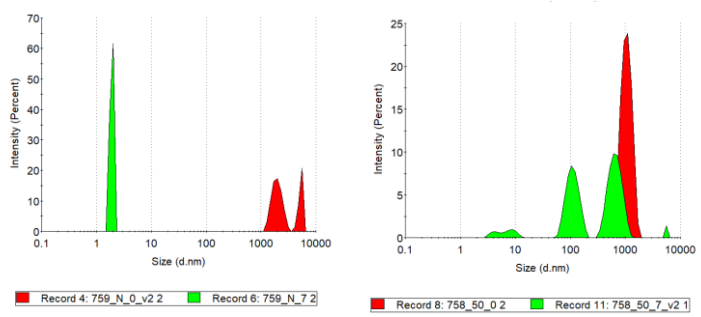
Bacteria



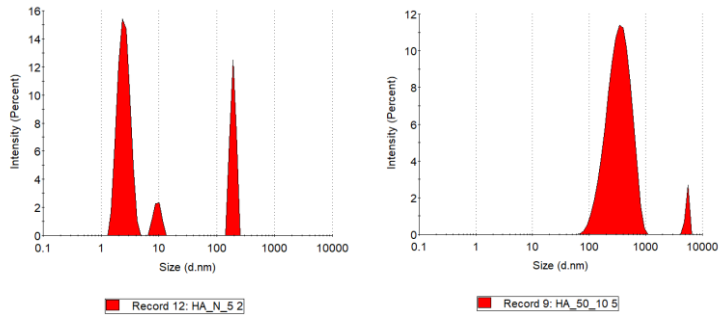
Archae



Laser light scattering

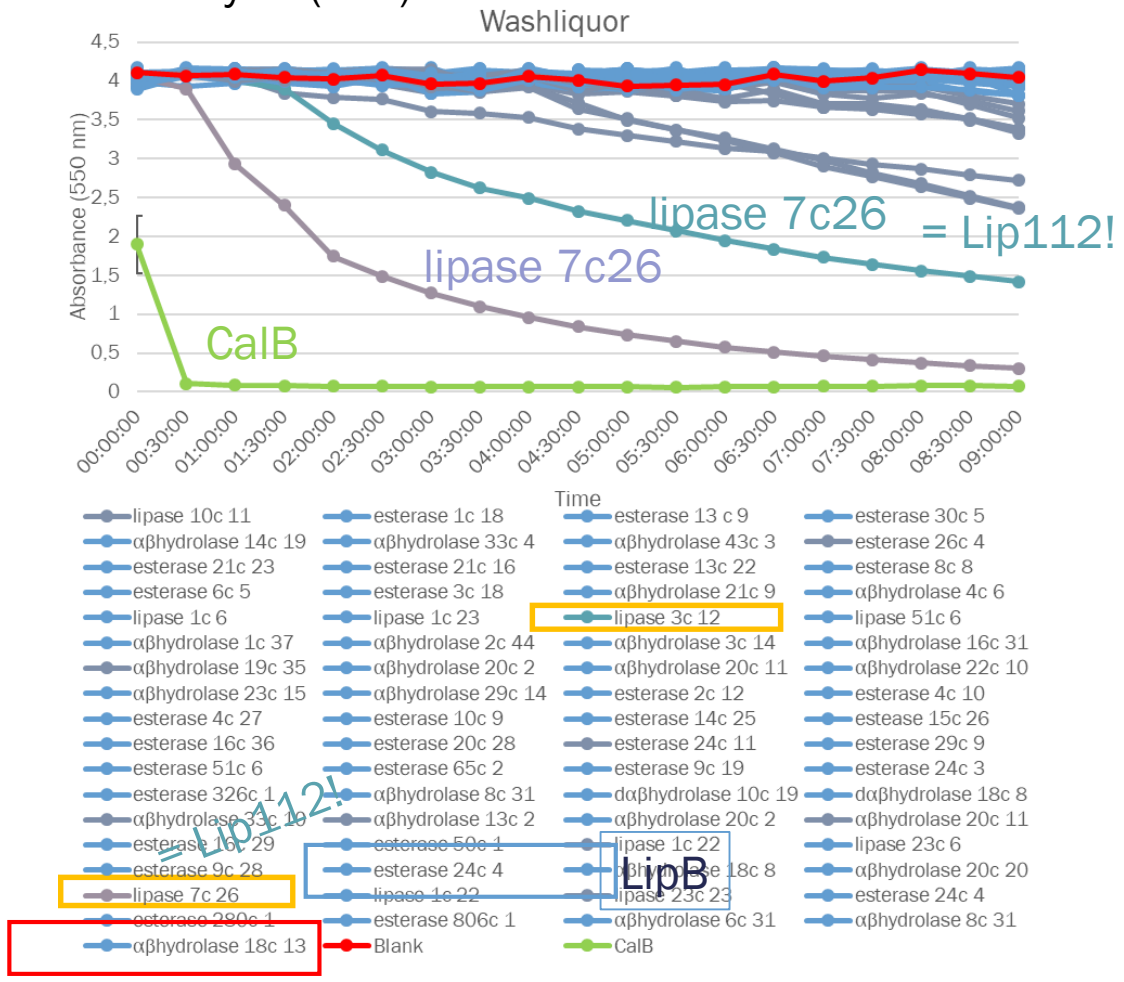


Laser light scattering



Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

Tributyrin (TBT)



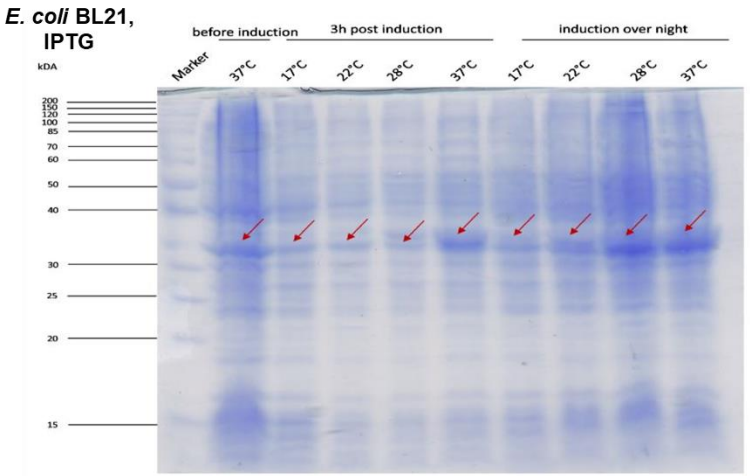
#27: 3_lipase_3c_12
#76 α/ß-hydrolase 18c13

	BL21	T7-Express	T7-Shuffle	Rosetta-Gami
#27				
#76	✓	✓		

Autoinduction medium
vs. LB + IPTG

Transformation → Optimization of expression conditions

#76 α/ß-hydrolase 18c13: 34.1 kDA



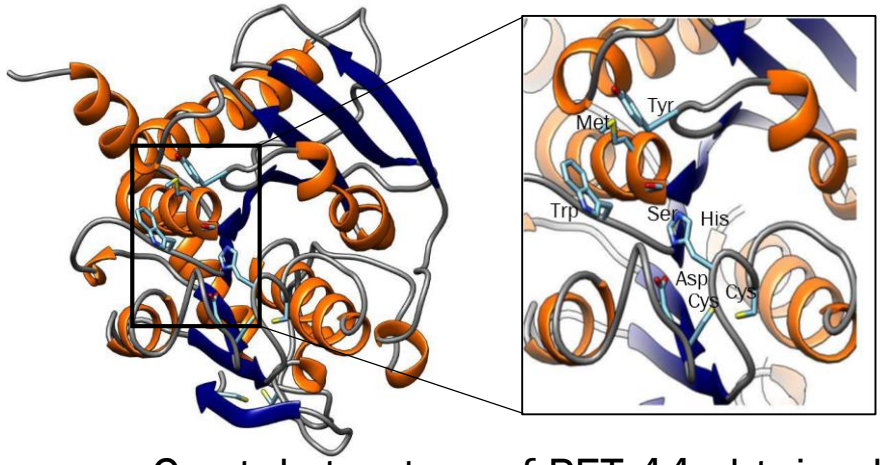
Task 4.4 Enzyme characterization for selecting those with manufacturers' specifications

PET44

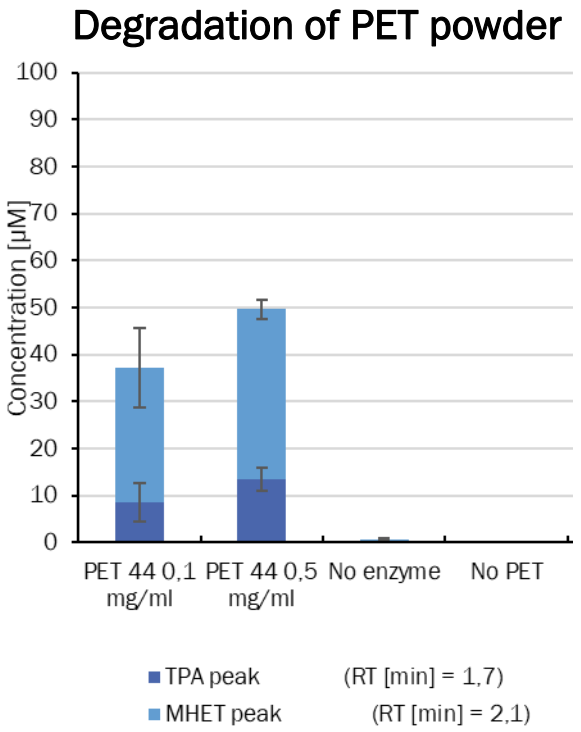
- Dienelactone hydrolase of species *Alkalilimnicola ehrlichii*
- PET44
  - 305 amino acids
  - 33 kDa
  - Temp. optimum 20-30°C
- Degrades PCL, TBT, BHET, Impranil DLN and PET

→ Appr. 40 - 50 µM of products after 4 days at 20°C on PET powder

- Lactonase (**δ**-Dodecalactone)



Crystal structure of PET 44 obtained



Task 4.5: Decision-making strategy for selecting lead enzyme candidates M6-M36

- Task Lead Partner – UDUS
- Participants: BANGOR, CSIC, UHAM, FHNW, IST-ID, EUC

Task 4.6: Design of multi-enzyme blends to process complex ingredient mixtures M12-M40

- Task Lead Partner – CSIC
- Participants: BANGOR, UDUS, UHAM, IST-ID

WP4 conclusions

- We conclude that CSIC has selected two best candidates for engineering and validations:
  - Lipase Lip9, of use for detergents and textiles
  - Lipase ID9, of use for detergents

- We conclude that CNR has a *Vibrio alginoliticus* isolate producing hyaluronidase (best candidate)
  - Either we plan fermentation at large
  - Or we wait for the results of gene synthesis of the two potential hyaluronidase genes, which is under progress

- We conclude that BSC and CSIC has the available PluriZyme and Biomimetic platform to produce proteases and oxidases.

WP4 further steps

- Clarify the candidates that have been / will be selected for scale up production and pre-industrial validations
- Each partner should select and discuss best candidates

- Please UHAM as WP leader