# Work Package 3: Activity-based bioprospecting for enzymes

Meeting #3

General Assembly – online – November 14 2022

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

| | | | |
|---|---|---|---|
| **Work package number** [9] | WP3 | **Lead beneficiary** [10] | 3 - BANGOR |
| **Work package title** | Activity-based bio-prospecting for enzymes | | |
| **Start month** | 1 | **End month** | 36 |

## Objectives

Entirely novel enzymes, which have no homologues in databases or with homology to known enzymes lower than ~20%, will escape the computational pre-screening in WP2. In order to circumvent this problem, WP3 combines three main pillars: bio-resources, technical capabilities for bio-resource handling and management, and activity-based multi-screens and Next Generation Sequencing (NGS), with a major objective: To screen for novel enzymes which, because their novelty, could not have been predicted using the BLAST, HMM and computational screens of WP2. To this end, the consortium has established a large collection of environmentally, geographically and taxonomically diverse bio-resources: 1. Cultured microbial isolates from (non)-extreme environments, many representing novel lineages; 2. Expression libraries from DNA from uncultivable microbial communities. 3. Genome sequences of cultivable microbial isolates; 4. Shotgun sequences of microbial communities; 5. Enzymes available in expression systems. The consortium has also at its disposal technical facilities and tools for: 1. Microbial handling and cultivation, including extremophiles, bioprospecting and sampling; 2. Handling and cloning DNA from uncultivable microorganisms; 3. High throughput screening supported by robotic and single cell manipulation workstations; 4. Multiple complementary analytics; 5. DNA sequencing (Illumina MiSeq and Oxford Nanopore instruments); 6. Bioinformatics analysis.

## Description of work and role of partners

**WP3 - Activity-based bio-prospecting for enzymes** [Months: 1-36]
**BANGOR**, CSIC, UHAM, UDUS, IST ID, CNR
In WP3 we propose 3 major tasks, through which we will implement sophisticated activity-based platforms to exploit available and new ad hoc bio-resources for entirely novel enzymes demanded by the detergent, textile and cosmetic sectors.

Task 3.1 Exploitation of the FuturEnzyme bio-resource collections M1-M24
Task Lead Partner – IST-ID
Participants: CSIC, UHAM, UDUS, BANGOR, CNR

Task 3.2 Sampling extreme environments for generating new microbial bio-resources M6-M30
Task Lead Partner – CNR
Participants: IST-ID

Task 3.3 Next Generation Sequencing for generating sequences of target enzymes M1-M36
Task Lead Partner – BANGOR
Participants: CSIC, UHAM, CNR

## Participation per Partner

| Partner number and short name | WP3 effort |
|---|---|
| 1 - CSIC | 5.00 |
| 3 - BANGOR | 17.00 |
| 4 - UHAM | 10.00 |
| 5 - UDUS | 4.00 |
| 6 - IST ID | 21.00 |
| 7 - CNR | 18.00 |
| **Total** | 75.00 |

## List of deliverables

| Deliverable Number[14] | Deliverable Title | Lead beneficiary | Type[15] | Dissemination level[16] | Due Date (in months)[17] |
|---|---|---|---|---|---|
| D3.1 | Bio-resources prepared and exchanged | 3 - BANGOR | Other | Confidential, only for members of the consortium (including the Commission Services) | 2 |
| D3.2 | Standard assays, analytics and calculations for monitoring enzymatic performance | 4 - UHAM | Report | Confidential, only for members of the consortium (including the Commission Services) | 2 |
| D3.3 | Set of 100 best clones, 10 isolates, and 10 enzymes shortlisted for sequencing or transfer to WP2 | 6 - IST ID | Other | Confidential, only for members of the consortium (including the Commission Services) | 10 |
| D3.4 | Sequence, activity, and stability datasets from best positive bio-resources | 3 - BANGOR | data sets, microdata, etc | Confidential, only for members of the consortium (including the Commission Services) | 18 |
| D3.5 | Set of new bio-resources to screen or sequence | 7 - CNR | Other | Confidential, only for members of the consortium (including the Commission Services) | 24 |
| D3.6 | Complete set of positive naïve screened enzymes and sequences and their datasets | 3 - BANGOR | Report | Confidential, only for members of the consortium (including the Commission Services) | 32 |

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

- A list of bio-resources available within the consortium have been prepared and exchange (D3.1 – month M2)
  - All partners implicated

# D3.1. BIO-RESOURCES PREPARED AND EXCHANGED

**ENZYMES AVAILABLE**
1353 entries, representing highly diverse enzymes relevant to FuturEnzyme, available in expression systems, from single (meta)genomes; the enzymes have been isolated and characterized for purposes others than those in FuturEnzyme, and will be now screened with project-relevant substrates and conditions.

**ISOLATES AVAILABLE**
1387 entries, representing psychrophilic, mesophilic, thermophilic, hyper-thermophilic, thermo-acidophilic, alcaliphilic, extreme halophilic, obligate anaerobic and facultative (micro)aerobic sulphur-respiring microorganisms. The collection includes strains growing at temperatures from 0° to 92°C, pH from 1.5 to 10.0, salinity up to 490 g/L, and pressure up to 50 Mpa.

**ISOLATES WITH GENOMES AVAILABLE**
197 entries, representing genomes from isolates representing lineages of (non)-extremophiles growing from 0 to 92°C, pH from 1.5 to 9.0, salinity up to 492 g/L, pressure up to 50 MPa.

**METAGENOMIC LIBRARIES**
28 entries, representing DNA material from communities inhabiting extreme environments (low pH from 1.1 to 4.4; high pH of 9.3-9.6; high salinity from 200 to 490 g/L; pressure up to 300 MPa; temperature up to 98°C) and non-extreme environments, including contaminated sites (close to neutral pH, low to moderate salinity (up to 50 g/l), temperatures from 4 to 30°C, up to 10.1 MPa).

**ENRICHMENT CULTURES**
41 entries, derived from samples originated from multiple locations and representing enriched microorganisms of at least 16 different genera.

**ISOLATES WITH PROVEN ACTIVITY**
55 entries.

**SHOTGUN METAGENOME SEQUENCES**
61 entries, corresponding to at least 16 different types of extreme and non-extreme environments.

These QR codes are confidential and available within the FuturEnzyme consortium. In order to increase the security, they have been blocked with a password (FuturEnzyme€01/06/2021). They will also be included in the private area of the FuturEnzyme website (www.futurenzyme.eu), in the section Shared material. This private area that serves as a repository for the project is accessible to the members of the consortium through user and password.



Submitted to the EU portal on 30th july 2021

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

- A number of assays for functional screens have been defined and shared (D3.2 – Month 2)
  - All partners implicated

# D3.2. STANDARD ASSAYS, ANALYTICS AND CALCULATIONS FOR MONITORING ENZYMATIC PERFORMANCE

**18x Protocols for DETERGENT APPLICATIONS**
4x pH shift liquid protocols for quantifying esterase-lipase activity
3x Liquid protocols for quantifying esterase-lipase activity with chromogenic esters
2x Liquid protocols for quantifying esterase-lipase activity with non-chromogenic esters
9x Agar plate protocols

**23x Protocols for TEXTILE APPLICATIONS**
7x Agar plate polyesterase screening assays
2x Agar plate protease protocols
1x Agar plate cellulase protocol
1x Agar plate oxidoreductase protocol
12x Liquid protocols

48 common and standardised protocols

**7x Protocols for COSMETIC APPLICATIONS**
4x Liquid colorimetric assays for hyaluronidase activity
1x Liquid analysis of hyaluronic acid oligosaccharides by HPAEC-PAD
1x Liquid analysis of hyaluronic acid oligosaccharides by SEC-ELSD
1x Agar assay for the determination of hyaluronidase activity

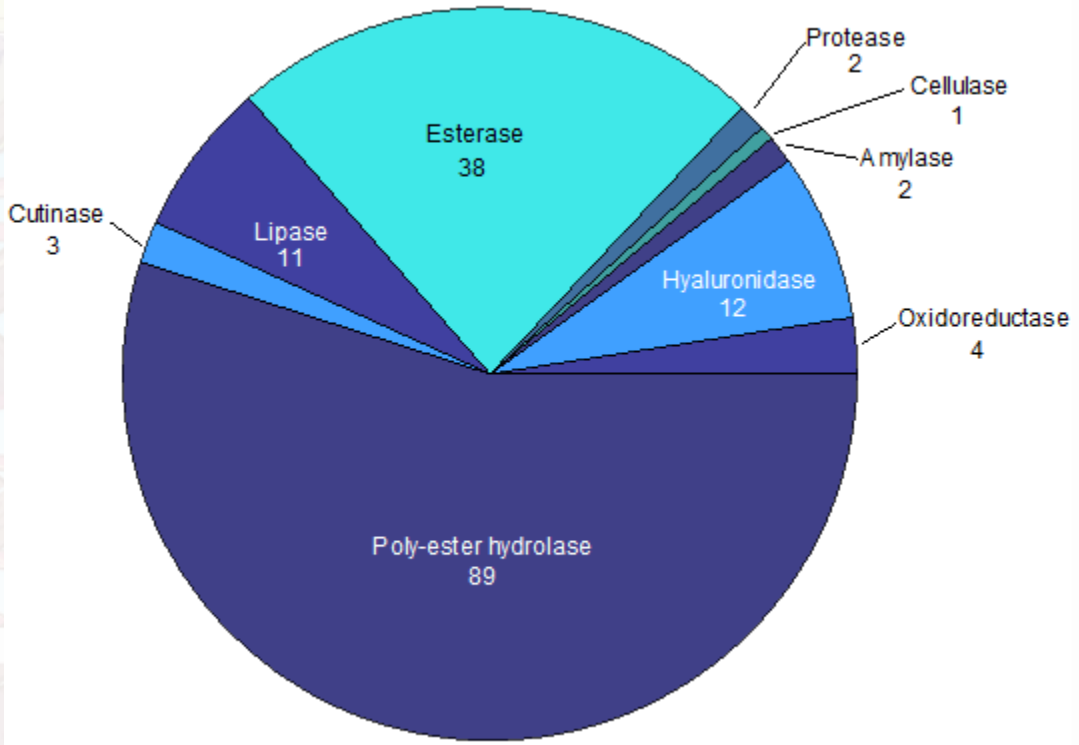


Submitted to the EU portal on 30th july 2021

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

- A list of best clones, isolates and enzymes have been prepared and shortlisted for sequences (D3.3 – month M10)
  - All partners implicated

# D3.3. 100 Best clones, 10 isolates, and 10 enzymes shortlisted for sequencing or transfer to WP2




Distribution by activity of the enzymes and microorganisms selected in this deliverable

The 154 prospects (up to 162, noticing that some isolates present different activities, so most probably several enzymes) have been settled apart out of 120 genomes from isolates, metagenomes from 47 microbial communities, 1200 microbial strains, 30 metagenome libraries and 500 enzymes (as mentioned in Task 3.1, Grant Agreement, Annex 1, part A).

# Isolates/Microorganisms

| | | | Number of isolates with activity/ies | First priority for (industrial partner/s) | Second priority for (industrial partner/s) |
|---|---|---|---|---|---|
| Oxidoreductase | | | 3 | Schoeller | Henkel |
| Hydrolase | Glucosidase | Hyaluronidase | 10 | Evonik | |
| | | Amylase | 1 | Scholler | Henkel |
| | Peptidase | Protease | 1 | Scholler | Henkel |
| | Ester-hydrolase | Esterase | 4 | Henkel/ Schoeller | |
| | | Lipase | 8 | Henkel/ Schoeller | |
| | | Cutinase | 3 | Henkel/ Schoeller | |

# Enzymes

| | | | | Number of enzymes with activity | First priority for (industrial partner/s) | Second priority for (industrial partner/s) |
|---|---|---|---|---|---|---|
| EC 1 | Oxidoreductase | Laccase, Cu-oxidase | | 1 | Schoeller | Henkel |
| EC 3 | Hydrolase | Glucosidase | Hyaluronidase | 2 | Evonik | |
| | | | Amylase | 1 | Scholler | Henkel |
| | | | Cellulase | 1 | Schoeller | |
| | | Peptidase | Protease | 1 | Scholler | Henkel |
| | | Ester-hydrolase | Esterase | 34 | Henkel/ Schoeller | |
| | | | Lipase | 3 | Henkel/ Schoeller | |
| | | | Poly-ester hydrolase | 89 | Schoeller | Henkel |

# D3.3. 100 Best clones, 10 isolates, and 10 enzymes shortlisted for sequencing or transfer to WP2

The following QR code directs to the full list of **candidate isolates**

The following QR code directs to the full list of **candidate enzymes**

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

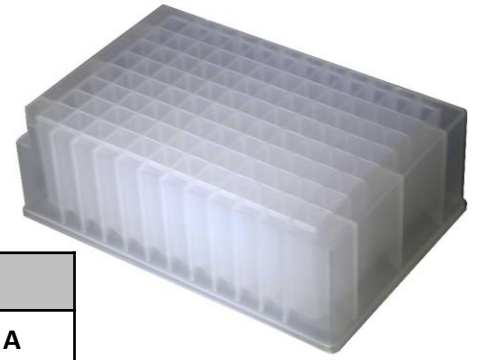
- Data set of sequence, activity and stability from best positive bio-resources (D3.4 – month M18) ***to be finalised now!***

# UDUS

Previosly reported

- WP3
  - ➢ Task 3.1 Exploitation of the FuturEnzyme bio-resource collections

✓ A set of 85 esterases and lipases including 16 cutinase-like enzymes from previous projects collected



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | LCC WCCG | Psab PE-H | Abo_LipA (CE02) | Paes TB045 (CE15) | lipA | Est24c11 | TBEc350 | Hyd8c31 | | Aku GDSL1 (CE17) | | Hyd20c2 | **A** |
| **B** | LCC | Paby PE-H | Hyd18c13 | Abo_LipC (CE09) | **EV** | TBEcIH8 | TBEc321 | Hyd18c8 | Hyd4c6 | Est8c8 | | Lip10c11 | **B** |
| **C** | PETase | Ppac PE-H | TBEc157 | Hyd13c2 | Aku Est3 (CE20) | | Est9c19 | Lip3c12 | Hyd3c14 | Abo_LipE (CE10) | | Est20c28 | **C** |
| **D** | Plit PE-H | Poce PE-H | TBEc310 | Abo_LipD (CE07) | Est9c28 | Hyd10c19 | TBEc314 | Aku Est2 (CE19) | Abo_LipG (CE11) | Abo_LipI (CE12) | | Est13c9 | **D** |
| **E** | Pbau PE-H | Paes PE-H Y250S | TBEc304 | Hyd33c4 | Lip1c6 | Est29c9 | Hyd20c11 | Paes TB035 (CE13) | 1,4-D#003 | Hyd22c10 | | Est30c5 | **E** |
| **F** | Ppel PE-H | Paes PE-H (CE16) | CycTB025 | Abo_Est3 (CE03) | MHETase | Est51c6 | Aku Est1 (CE18) | TBEc305 | Hyd23c15 | TBEc308 | **EV** | EstP | **F** |
| **G** | POIL-1 PE-H | Pxin PE-H | Hyd7c19 | **EV** | Dim-008 (CE01) | Paes TB037 (CE14) | Est16c36 | ED30 | Est24c4 | Hyd19c35 | Est65c2 | | **G** |
| **H** | Abo_Est7 (CE05) | Paes TB072 (CE24) | Dim004 | Abo_Est1 | Paes TB040 (CE22) | FScut | PaesTB074 (CE25) | Dim001 | Paes TB081 (CE23) | Abo Est2 (CE04) | Paes TB001 (CE21) | Abo Est12 (CE08) | **H** |

Table of ready to use UDUS/UHAM esterases & lipases at UDUS for FuturEnzyme activities

# UDUS

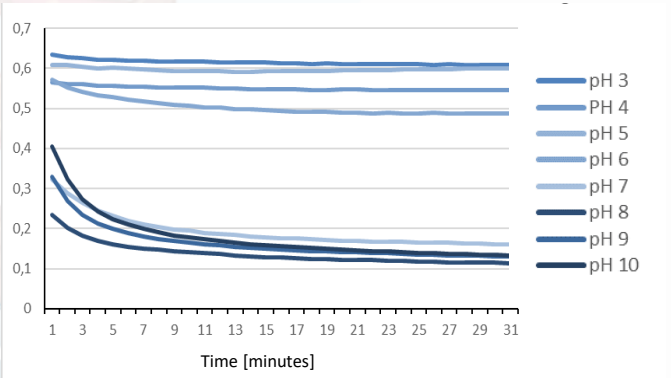
Previosly reported

- WP3
  - ➢ Task 3.1 Exploitation of the FuturEnzyme bio-resource collections

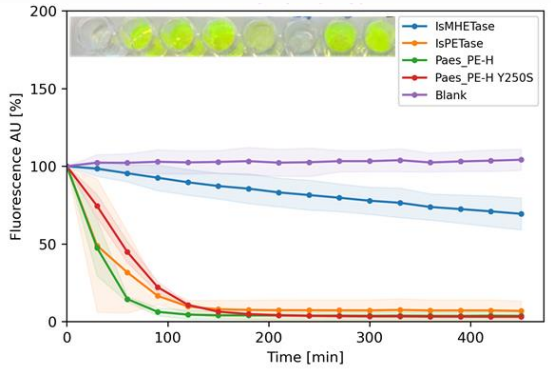
✓ A set of 88 esterases and lipases including 16 cutinase-like enzymes from previous projects collected

✓ MTP assay development

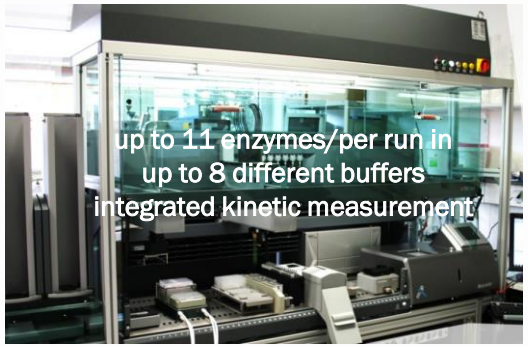
Turbidity measurements for cutinase characterization




pH indicator assay for hydrolysis of solid substrates




Implemented automated characterization



# UDUS

Previosly reported

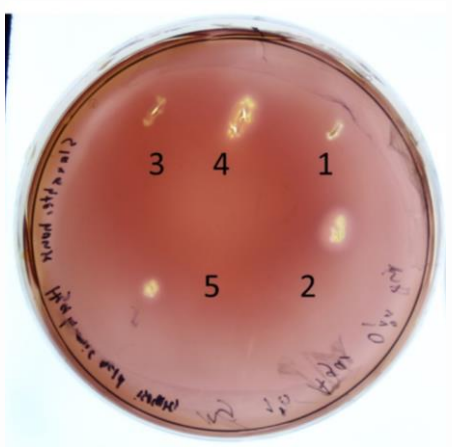
- WP3
  - ➢ Task 3.1 Exploitation of the FuturEnzyme bio-resource collections

✓Enrichment cultures of slaughterhouse metagenome bank with hyaluronic acid

➢Several strains with *in silico* put. hyaluronic acid lyases

➢Mainly from clade of y-gamma proteobacteria,
e.g., *Proteus* sp. or *Raoultella* sp.

M9 medium
+0.1% hyaluronic acid



# UDUS

- WP3 Deliverable 3.3 Resources

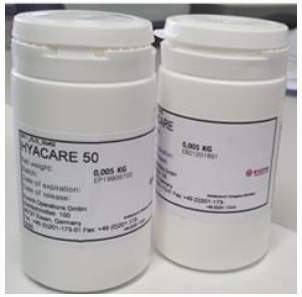
| | Source | Id-status | Genome status | Enzyme candidates |
|---|---|---|---|---|
| isolates showing hyaluronic acid hydrolysis | | | | |
| Proteus sp. | Slaughterhouse drain | Partial 16S rDNA sequence | Not sequenced, Sequence of related strains available | *In silico:* Chondroitin lyase Put. Hyaluronic acid AC Lyase |
| Isolate Hyal_hyal_UDUS 2 (Raoultella sp.) | Slaughterhouse drain | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Next step: *in silico* candidate enzyme identification ( BSC?) |
| Isolate Hyal_hyal_UDUS 3 (Raoultella sp.) | Slaughterhouse drain | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Next step: *in silico* candidate enzyme identification ( BSC?) |
| (Isolate Hyal_hyal_UDUS 4) (Spirosoma) | Slaughterhouse drain | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Next step: *in silico* candidate enzyme identification ( BSC?) |
| strains exceptionally enriched in esterases | | | | |
| Halopseudomonas aestusnigri | Oil polluted coast (spain) | Type strain | draft | 2 Polyesterases (1 confirmed), 12 confirmed additionally esterases |
| Halopseudomonas litoralis | Coastal waters (spain) | Type strain | Closed genome available. | 2 Polyesterases (1 confirmed) |
| Halopseudomonas oceani | Deep Sea | Type strain | draft | 2 Polyesterases (1 confirmed) |
| Halopseudomoans bauzanensis | Polluted industrial site soil (italy) | Type strain | draft | 2 Polyesterases (1 confirmed) |

# UDUS

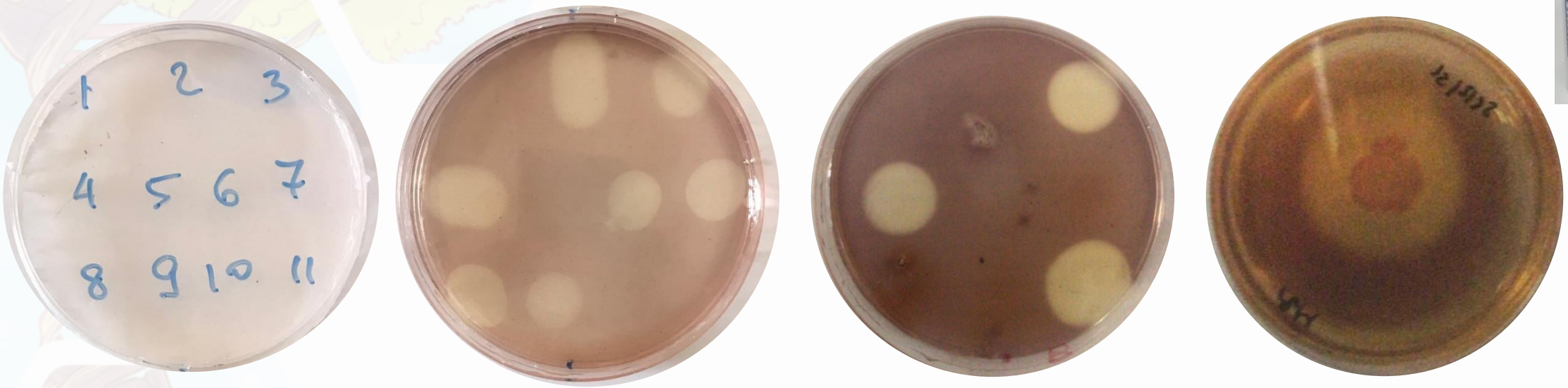
- WP3 Deliverable 3.3 Resources

| | Source | Id-status | Genome status | Enzyme candidates |
|---|---|---|---|---|
| isolates showing polyester hydrolysis | | | | |
| PEH_PUE UDUS1 | Deep Sea sediment | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Candidates identified by HMM (UHAM). Next step:cloning |
| PEH_PUE UDUS2 | Deep Sea sediment | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Candidates identified by HMM (UHAM). Next step:cloning |
| PEH_PUE UDUS3 | Deep Sea sediment | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Candidates identified by HMM (UHAM). Next step: cloning |
| PEH_PUE UDUS4 | Deep Sea sediment | Partial 16S rDNA sequence | Sequencing finished, draft (by Bangor) | Candidates identified by HMM (UHAM). Next step: cloning |
| PEH_PUE UDUS5 | Deep Sea sediment | Type strain | Sequencing finished, draft (by Bangor) | Candidates identified by HMM (UHAM). Next step: cloning |

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)




- Two hyaluronic-acid degrading isolates have been identified by CNR, when using EVO hyaluronic acid as substrate for screening




- *Halorhabdus* sp. SivX81 (genome sequenced)
- *Vibrio alginolyticus* from anoxic sediments of meromictic brackish Lake Faro, Messina

# List of hydrolytic halo- and halonatronoarchaea

| Strain | Lakes | Affiliation | Substrate | Activity | Growth |
|---|---|---|---|---|---|
| H-hyl | Cl | Halobacteria (new genus?) | Hyaluronic acid (HA) | + | + |
| RMX81 | Cl | Halorhabdus sp. | Coco oil, xylan. HA | + | + |
| RMX62 | Cl | Halorhabdus uthaensis | Coco oil, skim milk, xylan, HA | + | + |
| Siv8X | Cl | Halorhabdus uthaensis | Coco oil, skim milk, xylan HA | + | + |
| HArcel-Eu2 | Cl | Halomicrobium sp. | Hyaluronic acid, cellulose | + | + |
| HArcel2** | Cl | Halosimplex sp. | Cellulose | + | + |
| HArcel3** | Cl | Halomicrobium sp. | Cellulose | + | + |
| Harc-L1 | Cl | Halobacteria (unidentified) | Olive oil | + | + |
| Harc-L2 | Cl | Halobacteria (unidentified) | Olive oil | + | + |
| BNX81 | Cl | Halococcoides cellulosivorans | Cellulose, xylan HA | + | + |
| LCL711 | | Halorhabdus sp. | Xylan, hyaluronic acidHA | + | + |
| | | | | | |
| AB-hyl1 | SL | Paracoccus sp. | Hyaluronic acid (HA) | + | + |
| AArcel7 | SL | Natrarchaeobius sp. | Hyaluronic acid (HA) | + | - |
| AArc-St1-1* | SL | Natranaeroarchaeum aerophilum | Hyaluronic acid (HA) | + | - |
| AArc-L1 | SL | Natrarchaeobaculum aegyptiacus | Olive oil | + | + |
| AArc-L2 | SL | Natronolimnohabitans innermongolicus | Olive oil | + | + |
| AArc-LBj | SL | Halobacteria (unidentified) | Olive oil | + | + |
| | | | | | |

---

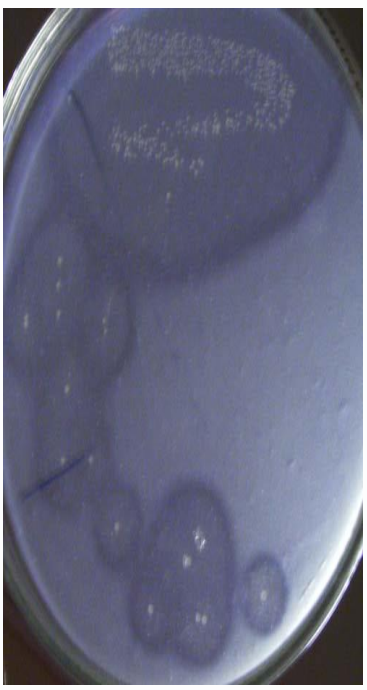
S - soda lakes; Cl - chloride lakes;

* - extremely high carotenoid content - suitable for production?

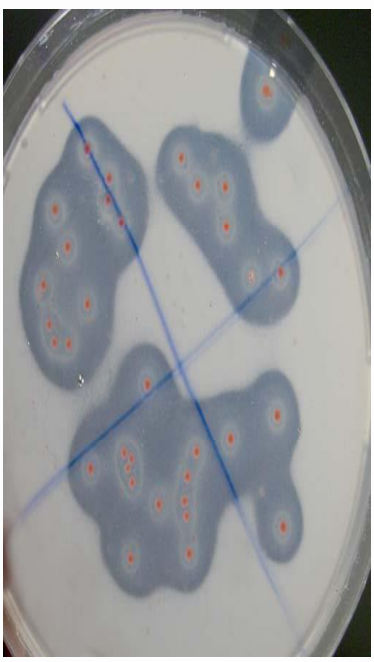
** - for genomes

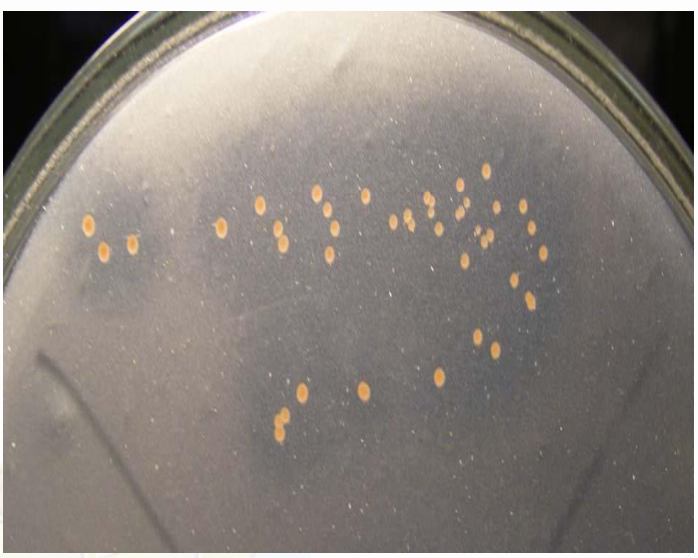
Bold – genome sequenced

AArc-L1



AArc-LBj

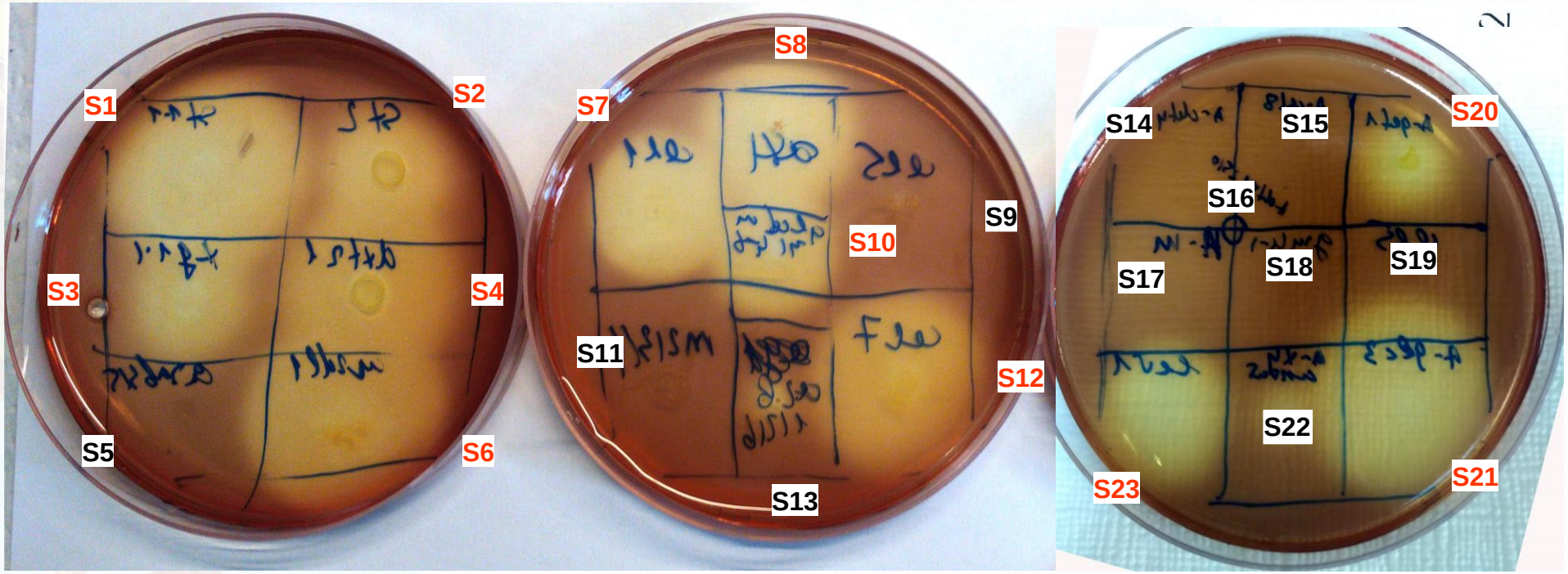




AArc-L2

Lipase activity in colonies of natronoarchaea

# Natronoarchaeal HA+ isolates grown at 4 M $Na^+$/pH 9.5 (Hyaluronic acid+ye) / 37°C/ 9d)







**Soda lake natronoarchaea**

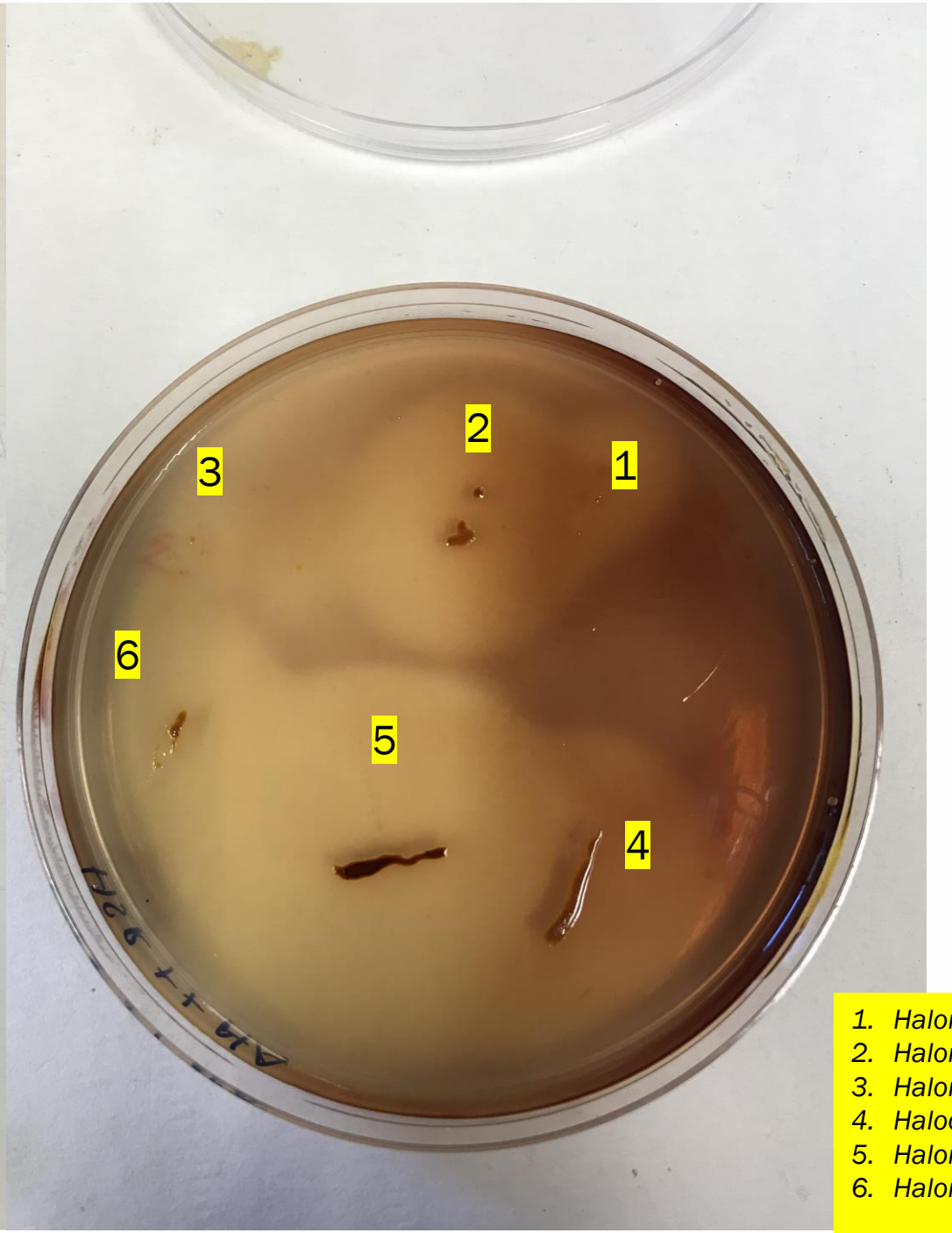
S1: AArc-St1-1 (amylolytic)
S2: AArc-St2 (amylolytic)
S3: AArc-xg1-1 (xyloglycan)
S4: AArc-dxtr1 (dextran)
S5: AArc-arb3/5 (arabinan)
S6: AArc-curdl1 (curdlan)
S7: AArcel1 (*Natronolimnobius*; cellulo-xylan)
S8: AArc-ax1(*Natronolimnobius*; arabinoxylan)
S9:AArcel5 (*Natronobiforma*; cellulo-xylan)
S10: AArc-glctm3/4/8 (*Natronococcus*; glct-mannan)
S11:AArc-m2/3/4 (mannan-cellulo)
**S12**: AArcel7 (chitin)
S13: AArc-arb1/2/6 (*Natronolimnobius*; arabinan)

**S13**

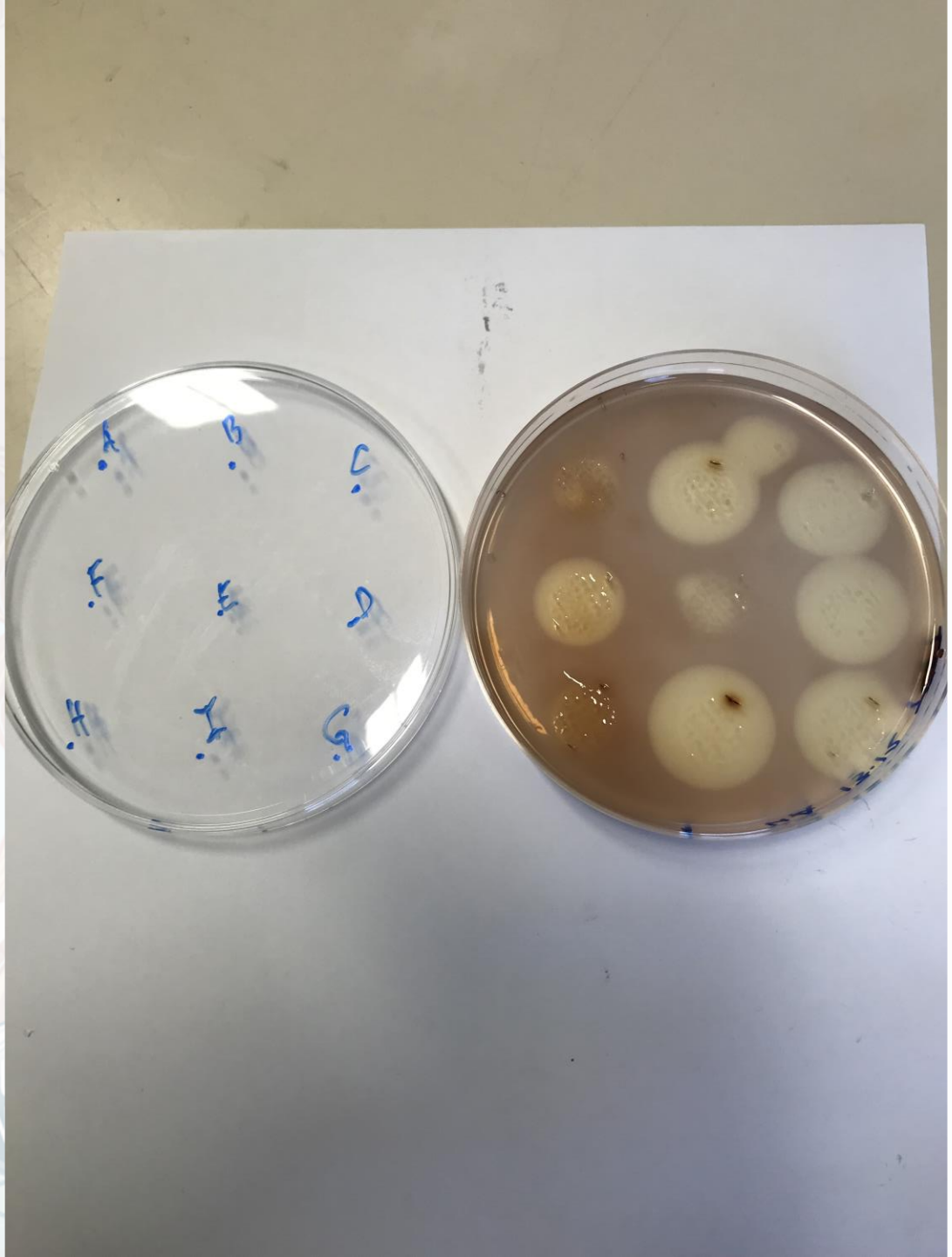
S14: AArcht4 (*Natrarchaeobius chitinivorans*)
S15: AArcht8 (*Natrarchaeobius chitinivorans*)
S16: AArcht-Sl (*Natrarchaeobius chitinivorans*)
S17-19: Mannan-utilizing natronoarchaea
S20:AArc-glct1 (galactan)
**S21**: AArc-glc3 (*Natronorubrum tibitense*; glycogen)
S22: AArc-X4 (*Halomicrobium* sp; cellulo)
S23: AArc-lev1 (levan)[~Aarc-St1-1]

Only **AArcel7** showed moderate growth with Hyl in liquid culture without ye. But it faded after second transfer
Colony growth: S1, S2, S3, S4, S6(weak), S7, S8, S9(w), S10, S11, **S12,** S13; Cl6


*Halorhabdus* sp. LCL711




1. *Halomicrobium* sp. LC1Hm
2. *Halorhabdus uthaensis* Siv8X
3. *Halomicrobium* sp. Harcel-Eu2
4. *Halococcoides cellulosivorans* BNX81
5. *Halorhabdus* sp. RMX81
6. *Halorhabdus uthaensis* RMX62




*A. Haloferax lucertense* SVX82
*B. Halosimplex sp.* HArcel2
*C. Halomicrobium* sp. Harcel3
*D. Halobacteria* (new genus) H-hyl
*E. Haloferax alexandrinus* BNX82
*F. Halorhabdus* sp. KCL-HA6
*G. Halococciodes* sp. BariCL
H. *Haloferax* sp. SVXCL
I. *Halorhabdus* sp. KCL5

Hyaluronidase activity by agar-diffusion test of haloarchaeal cultures grown with cellobiose + hyaluronate for one week. Fraction of 50 µl of supernatant was placed on filter discs and incubated on the plates with hyaluronic acid (400 mg $l^{-1}$) at pH 7.5, 4 M total $Na^+$, 37 °C, 24h;

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

- Three lipases have been identified by **CSIC**, when screening two fosmid libraries made at BANGOR using tributyrin, olive oil, egg yolk, cocoa and coconut
  - *2 clones from D2 library (bone [turkey femur]-degrading microbiome; 11.12.2017;* Byfjorden (60,238185N, 5,181210E) were found positive for *tributyrin, egg yolk and cocoa*
    - *D2 pCCFOS fosmid library has a titre of 9000 clones max.*
  - *1 clone from I3 library (bone [cow tibia]-degrading microbiome; 11.12.2017;* Byfjorden (60,238185N, 5,181210E) were found positive for *tributyrin, egg yolk, olive oil and cocoa*
    - *I3 pCCFOS fosmid library has a titre of 2000 clones max.*

Previosly reported

# Task 3.2 Sampling extreme environments for generating new microbial bio-resources

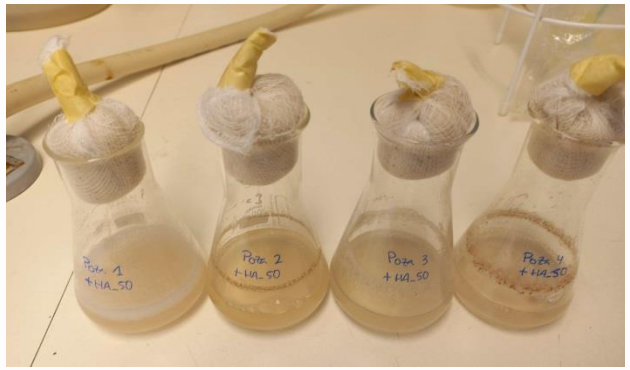
- sampling for new bio-resources; identifying novel microbes and enzyme activities; screening our microbial collection for efficient enzymes

IST-ID
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento

## New samples since June (M12-M18)

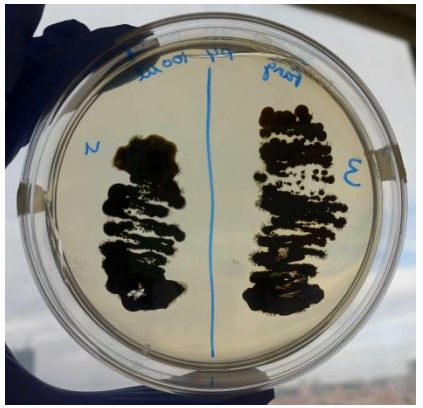
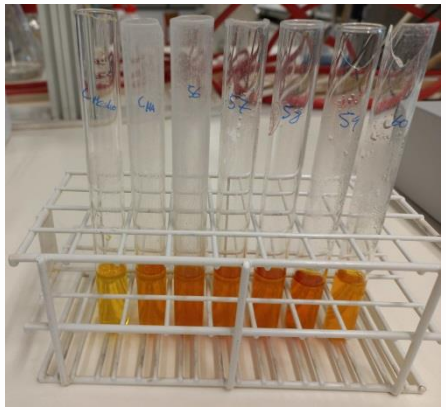
Graciosa island, the Azores, PT



Samouco salterns, PT



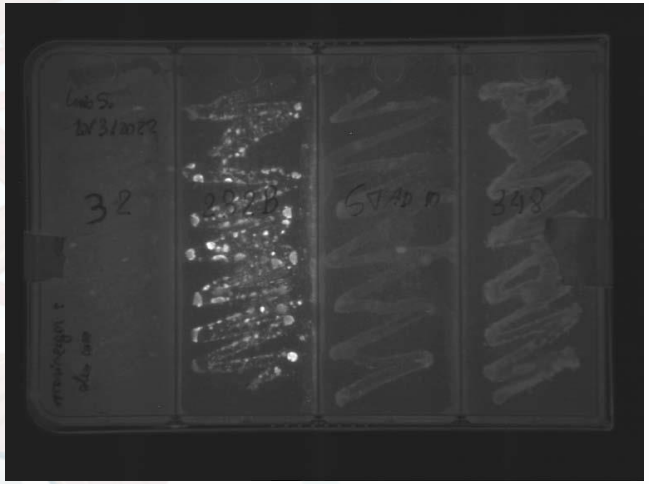
# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections

- sampling for new bio-resources; identifying novel microbes and enzyme activities; screening our microbial collection for efficient enzymes

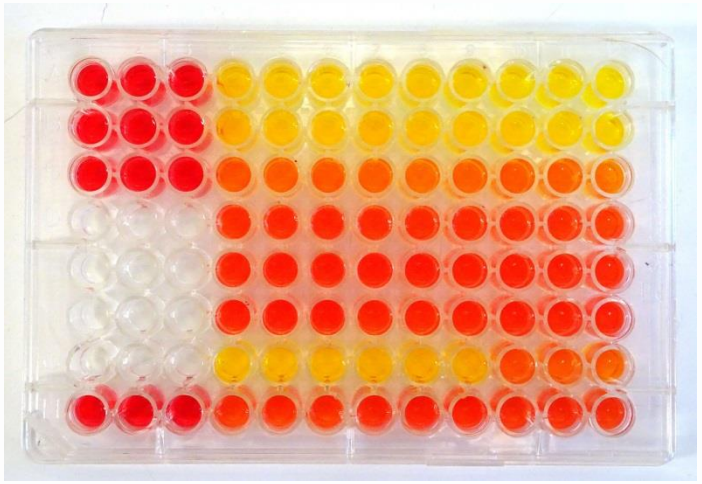
IST-ID
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento

## Lipases/Esterases

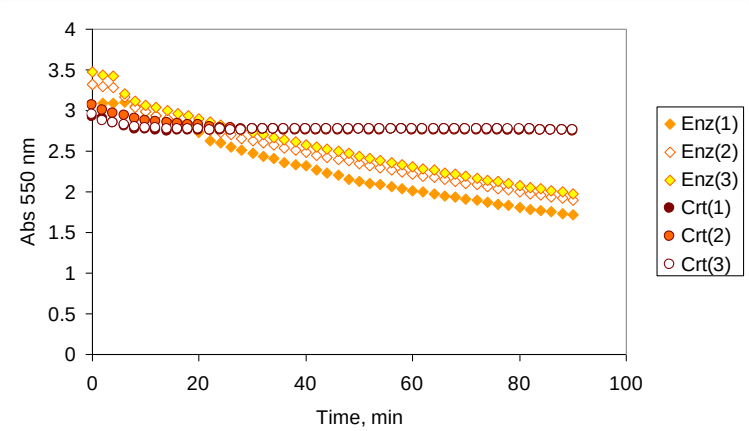
| Bacterial collection | Tributyrin or Tween 80 | Cononut oil | Palm oil | Olive oil |
|---|---|---|---|---|
| Existing | 40* | 5 | 0 | 6 |
| FuturEnzyme (new) | 7 | 12 | 1 | 16 |



Screening isolates with lipase activity



Effect of olive oil concentration during bacterial growth on lipase activity



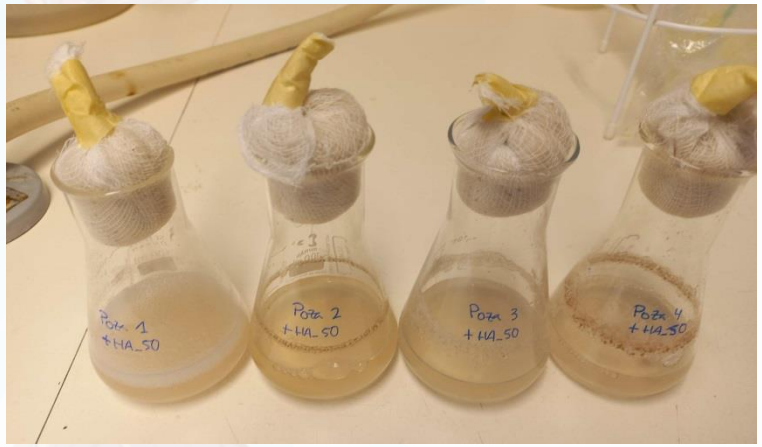
# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections

- sampling for new bio-resources; identifying novel microbes and enzyme activities; screening our microbial collection for efficient enzymes

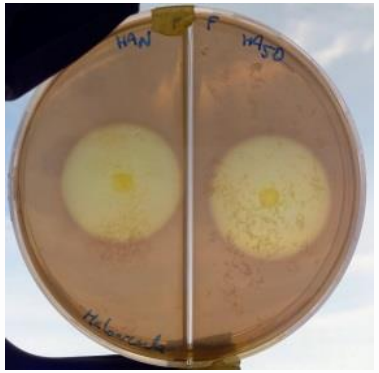
IST-ID
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento

## Hyaluronidases

| Bacterial collection | HA (Hyacare) | HA5 (Hyacare) |
|---|---|---|
| Existing | 2* | 2* |
| FuturEnzyme (new) | 14 | 14 |

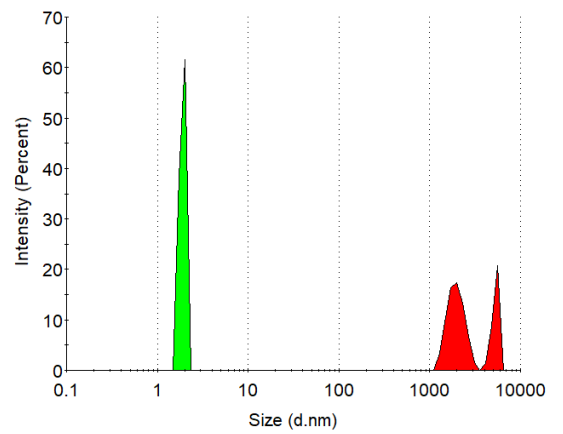



Enrichment cultures with HA and HA50

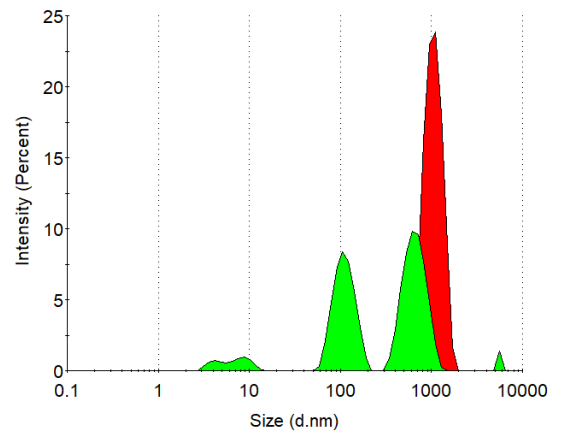



Screening

Laser light scattering




*Stutzerimonas stutzeri*




*Microbacterium oxydans*

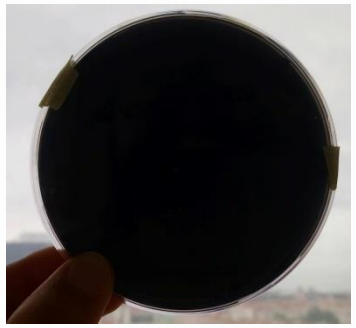
# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections

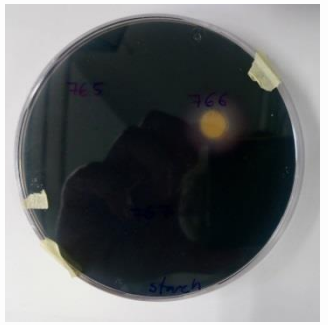
- sampling for new bio-resources; identifying novel microbes and enzyme activities; screening our microbial collection for efficient enzymes

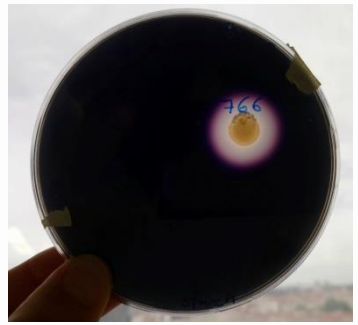
IST-ID
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento

## Amylases

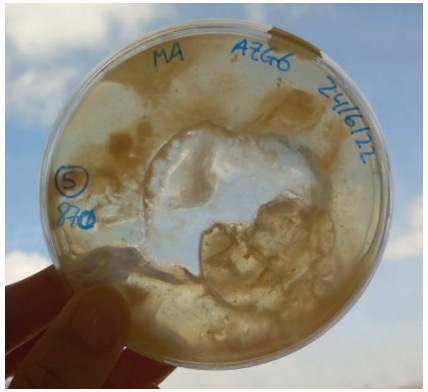
| Bacterial collection | Starch |
|---|---|
| Existing | 48* |
| FuturEnzyme (new) | 7 |







## Agarases



# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections

- sampling for new bio-resources; identifying novel microbes and enzyme activities; screening our microbial collection for efficient enzymes

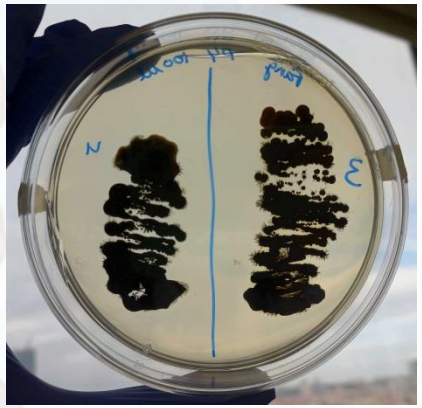
IST-ID
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento

## Other activities

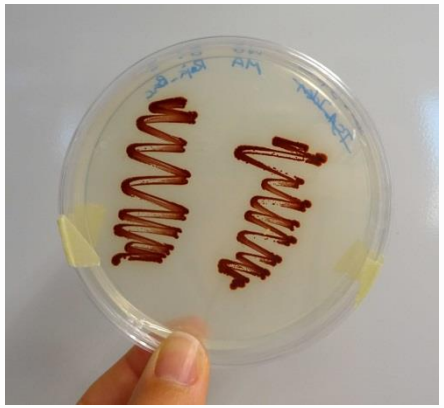
| Bacterial collection | Protease | Inulinase | Transaminase |
|---|---|---|---|
| Existing | 48* | 46* | 7* |

* Mostly *Bacillus* sp.; presented in MS9

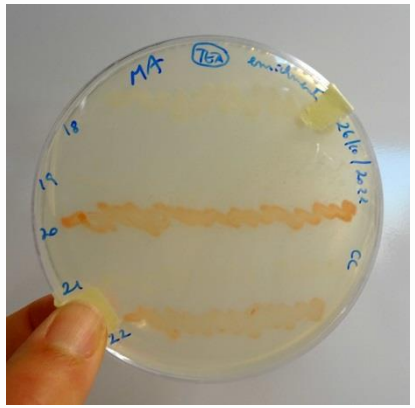
## Production of interesting compounds



Melanin by
*Neophaeotheca triangularis*



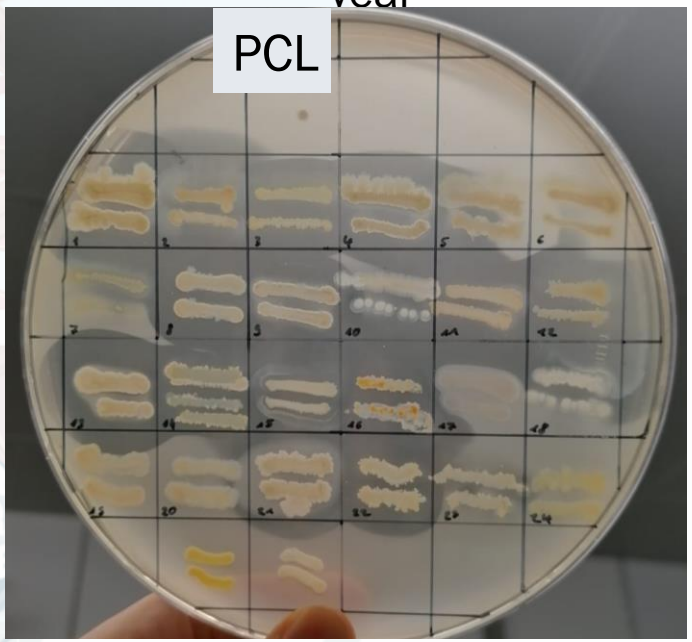
Prodiginines by
*Serratia* sp.

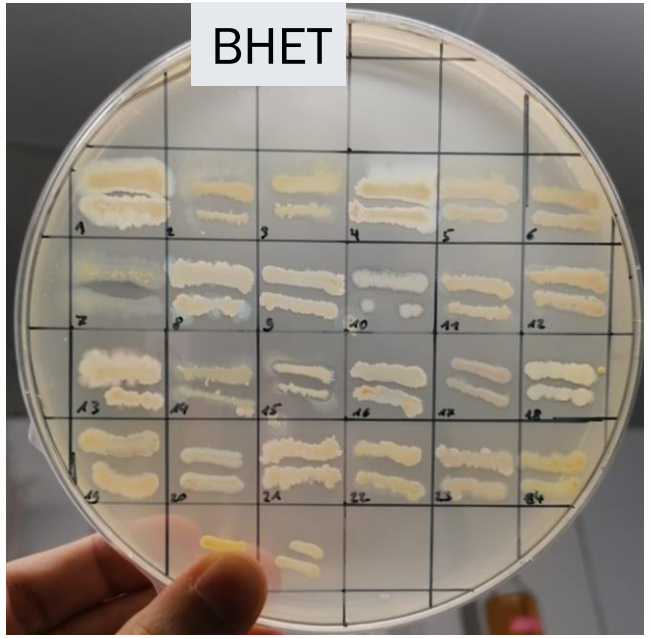


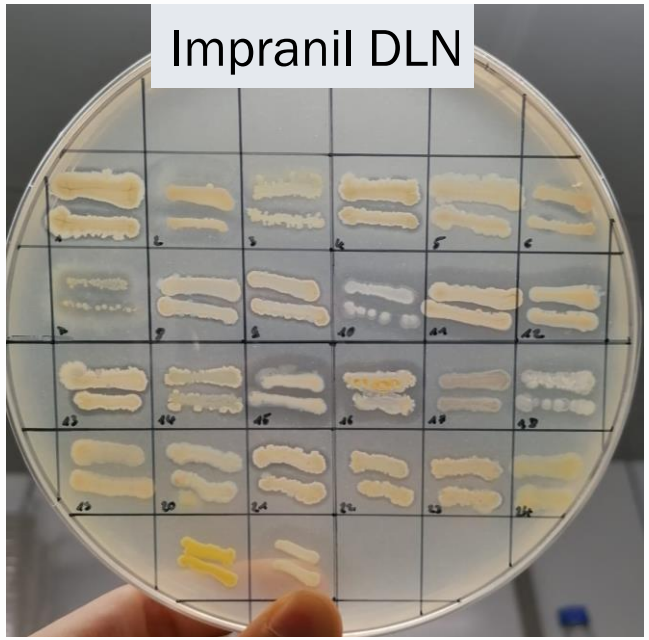
Carotenoids by several species

# Enrichments for polymer-degrading microorganisms

Enriched in M9 medium, anaerobic + aerobic, 28°C, over up to 1 year



# Enrichments for polymer-degrading microorganisms

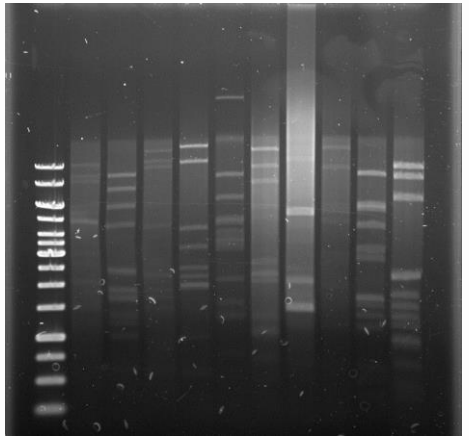
## 16 S Sequencing results of isolated single strains

| | |
|---|---|
| 1 | *Bacillus aeirus* |
| 2 | *Stutzerimonas stutzeri strain* ATCC 14405 |
| 3 | *Bacillus* sp. |
| 4 | *Pseudomonas aeruginosa* PA14 |
| 5 | *Achromobacter sp.* |
| 6 | *Pseudomonas mendocina strain* NEB698 |
| 7 | *Bacillus altitudinis* |
| 8 | *Pseudomonas plecoglossicida* |
| 9 | *Pseudomonas putida* |
| 10 | *Pseudomonas mendocina* |
| 11 | *Pseudomonas putida strain* KT2440 |
| 12 | *Priestia megaterium* |
| 13 | *Pseudomonas proteolytica* |
| 14 | *Bacillus altitudinis* |
| 15 | *Stutzerimonas stutzeri strain* ATCC 14405 |
| 16 | *Pseudomonas indoloxydans* |
| | *Acinetobacter sp. NyZ410* |

## Fosmid library of *Rhodococcus fascians*

photo: DMSZ

600 clones



30-37 kb inserts

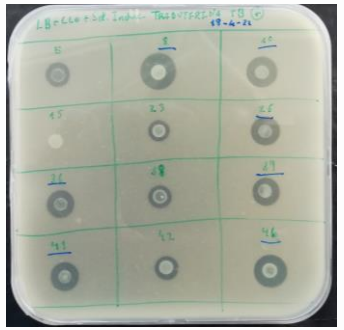
| Sub-strate | Degrading Colonies |
|---|---|
| DLN | 2 |
| BHET | 2 |
| PCL | 1 |
| TBT | many |

to be verified…

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

- 34 lipases have been identified by CSIC, when screening 5 fosmid libraries made at BANGOR using tributyrin, olive oil, egg yolk, cocoa and coconut

| # Site-library | Nr. Clones | Nr of positives |
|---|---|---|
| D2 library (bone [turkey femur]-degrading microbiome; 11.12.2017; Byfjorden (60,238185N, 5,181210E) | 9000 | 2 |
| I3 library (bone [cow tibia]-degrading microbiome; 11.12.2017; Byfjorden (60,238185N, 5,181210E) | 2000 | 1 |
| MedSea clone library (Ancona port, Italy, 43°37′N; 13°30′15″E) | 10300 | 7 |
| Acid mine dranage system (Spain; 43°15′47″N, 5°46′9″W) | 11600 | 13 |
| TB (Thermophillic Bacteria) (mix genomes) | 11800 | 11 |
| TOTAL | 44700 | 34 |



Previosly reported

# Task 3.1 Exploitation of the FuturEnzyme bio-resources collections (M1-M24)

Screening of Ischia hot vents BBP-enrichment fosmid library (Ischia,Italy) (10000 clones max) identified:

- 12 fosmid clones positive for tributyrin, 2 fosmids clones positive for tributyrin, coconut oil, palm oil
- 16 fosmid clones with amylase activity for starch
- 3 fosmid clones with laccase activity for syringol and 3 fosmid clones with protease activity for skim milk

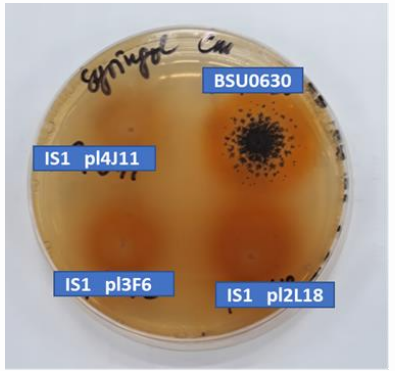
Screening of soil fosmid library (9000 clones max) identified:

- 16 fosmid clones were positive for tributyrin

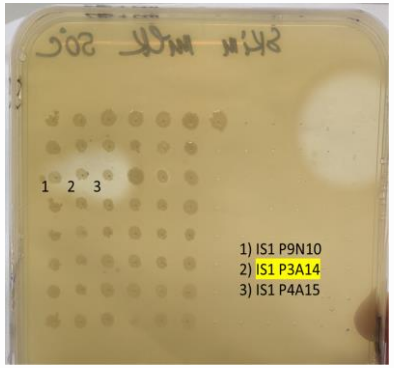
Previosly reported

tributyrin


starch




syringol




skim milk

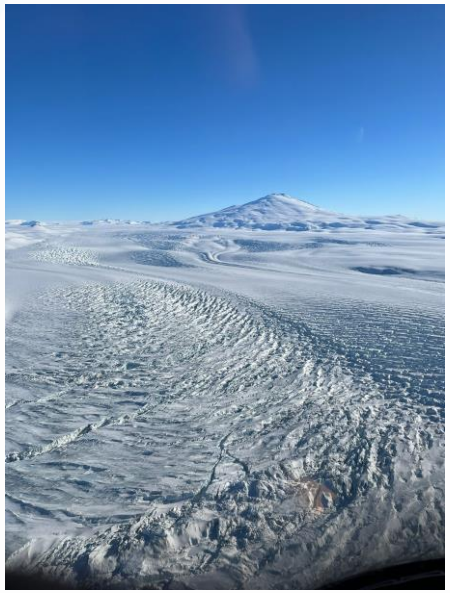
# Task 3.2 Sampling extreme environments for generating new microbial bio-resources M6-M30

- Sampling activities are planned to generate new isolates and sequences to feed WP2/WP4 ***in progress***

Milestone 11: *"The first sampling campaign completed"*

# Task 3.2 Sampling extreme environments for generating new microbial bio-resources M6-M30

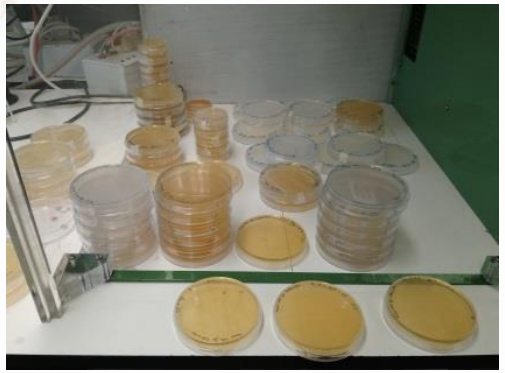
- Sampling activities are planned to generate new isolates and sequences to feed WP2/WP4 ***in progress***

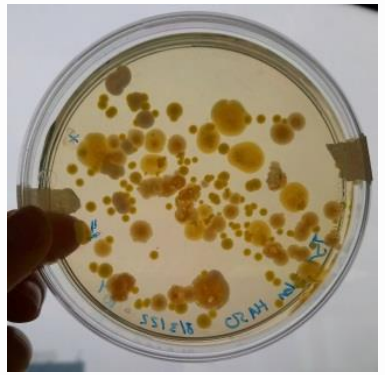



# Task 3.2 Sampling extreme environments for generating new microbial bio-resources

- sampling for new bio-resources; identifying novel microbes and enzyme activities; screening our microbial collection for efficient enzymes

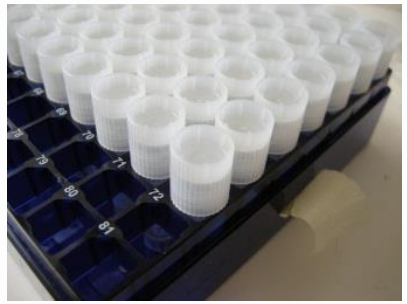
Rock pool at Guincho, Portugal






Previosly reported

Sherlock® Microbial ID System



Screening

# Task 3.3 Next Generation Sequencing for target enzymes M1-M36

- *Thermoleophilum album* – thermophilic, obligate hydrocarbonoclastic, high-GC Gram+.
  Genome sequenced at Bangor
  26 genes were cloned, **5** soluble esterases purified, **2** lipases were found active with C14-C18 pNp-esters

Previosly reported

# Task 3.3 Next Generation Sequencing for generating sequences of target enzymes M1-M36

PRIFYSGOL BANGOR UNIVERSITY

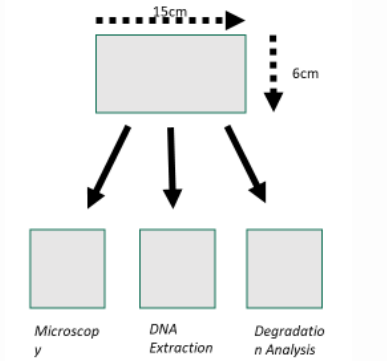
Sequences available for enzyme mining:

- 39 treatments/timepoints of microbiomes of colonisers of plastics (HDPE, LDPE, PP, PE and PET) in a transition:
  -wastewater treatment plant effluent
  -river water
  -brackish water
  -seawater (mesocosm) at the School of Ocean Sciences, Bangor University

Sequenced by Bangor (NERC 'Plastic Vector' project)
**>250 Gb** data available for enzyme mining **in** FuturEnzyme

Previosly reported

| Water | Time |
|---|---|
| Effluent | 24 Hours |
| Fresh | 16 Hours |
| Brackish | 18 Hours |




# Task 3.3 Next Generation Sequencing for generating sequences of target enzymes M1-M36

Menai Straits surface seawater an the St George Pier, School of Ocean Sciences, Bangor University
Lignin enrichment (shotgun metagenome sequenced at Bangor)
18 genes from 6 enzyme families with highest coverage were cloned

Previosly reported

# Task 3.3 Next Generation Sequencing for generating sequences of target enzymes M1-M36

- 30 treatments/timepoints of microbiomes from anaerobic bioreactors set up from Landfill in Penhesgyn Recycling Centre, Anglesey Natural Resources Wales (co-participant) 200 Gbp sequencing data

  **11** amylases (most-abundant in metagenomic reads) (s. overleaf) cloned, **5** expressed soluble, 1 found active with starch substrates

  8 Hyaluronidase cloned, **6** expressed soluble.

Previosly reported

# Task 3.3 Next Generation Sequencing for generating sequences of target enzymes M1-M36

Bangor: Sequencing isolates for UDUS. Sequencing strains of rhodococci purchased at DSMZ

Your Samples

| Barcode | Type | Coverage | Customer's Ref | Taxon | Current Queue (Status) |
|---|---|---|---|---|---|
| 238336 | WGS_DNA | 30x | Sample1 | Spirosoma endbachense | Bioinformatics |
| 238337 | WGS_DNA | 30x | Sample2 | Raoultella ornithinolytica | Bioinformatics |
| 238338 | WGS_DNA | 30x | Sample3 | Raoultella ornithinolytica | Bioinformatics |
| 238339 | WGS_DNA | 30x | Sample4 | Pseudomonas knackmussii | Bioinformatics |
| 238340 | WGS_DNA | 30x | Sample5 | Microbacteriu:n profundi | Bioinformatics |
| 238341 | WGS_DNA | 30x | Sample6 | Escherichia fergusonii | Bioinformatics |
| 238342 | WGS_DNA | 30x | Sample7 | Phenylobacterium falsum | Bioinformatics |
| 238343 | WGS_DNA | 30x | Sample8 | Erythrobacter pelagi | Bioinformatics |
| 238344 | WGS_DNA | 30x | Sample9 | Rhodococcus fascians | Bioinformatics |
| 238345 | WGS_DNA | 30x | Sample11 | Rhodococcus rhodochrous | Bioinformatics |
| 238346 | WGS_DNA | 30x | Sample12 | Rhodococcus rhodochrous | Bioinformatics |
| 238347 | WGS_DNA | 30x | Sample13 | Rhodococcus sp. | Bioinformatics |
| 238348 | WGS_DNA | 30x | Sample14 | Rhodococcus sp. | Bioinformatics |
| 238349 | WGS_DNA | 30x | Sample15 | Rhodococcus rhodochrous | Bioinformatics |

UDUS

Bangor (DSMZ strains)

Complete on November 7, 2022

# Task 3.3 Next Generation Sequencing for generating sequences of target enzymes M1-M36

Bangor:

Sequencing of isolates for CNR Messina. Hyaluronidase and other GH-positive.

## Your Samples

| Barcode | Type | Coverage | Customer's Ref | Taxon | Current Queue (Status) |
|---|---|---|---|---|---|
| 233161 | WGS_DNA | 30x | PG22_01 | Halorhabdus | Complete |
| 233162 | WGS_DNA | 30x | PG22_02 | Halorhabdus | Complete |
| 233163 | WGS_DNA | 30x | PG22_03 | Halorhabdus | Complete |
| 233164 | WGS_DNA | 30x | PG22_04 | Halorhabdus | Complete |
| 233165 | WGS_DNA | 30x | PG22_05 | Halorhabdus | Complete |
| 233166 | WGS_DNA | 30x | PG22_06 | Halorhabdus | Complete |
| 233167 | WGS_DNA | 30x | PG22_07 | Halorhabdus | Complete |
| 233168 | WGS_DNA | 30x | PG22_08 | Halorhabdus | Complete |
| 233169 | WGS_DNA | 30x | PG22_09 | Halorhabdus | Complete |
| 233170 | WGS_DNA | 30x | PG22_10 | Vibrio alginolyticus | Complete |
| 233171 | WGS_DNA | 30x | PG22_11 | Vibrio alginolyticus | Complete |
| 233172 | WGS_DNA | 30x | PG22_12 | uncultured gamma proteobacterium | Complete |

Haloarchaea (CNR)

Marine gamma-proteo (CNR)

Complete on July 1, 2022

**Task 3.3 Bio-resources** (Bangor) (new )

**62** genes synthesized since last meeting in Madrid (in addition to **37** previously synthesized at Bangor) for:

**1**. 8 glycosyl hydrolases (new HA-candidates from sequenced genomes of **Partner CNR))**

2. **19** Lipases, esterases, laccases, peroxidases-catalases from *Oleiphilus messinensis* ME102$^{T}$
3. **9** hydrolases from Parys Mt hyperacidic (pH 1.5) fosmid library
4. **4** MCO, peroxidases from PP and LDPE colonisers
5. **22** LipEst, MCO, peroxidases/catalases from 1-week PET colonisers shotgun metagenome-sequencing data (Menai Strait seawater mesocosm)

# Deliverables

List of deliverables

| Deliverable Number[14] | Deliverable Title | Lead beneficiary | Type[15] | Dissemination level[16] | Due Date (in months)[17] |
|---|---|---|---|---|---|
| D3.1 | Bio-resources prepared and exchanged | 3 - BANGOR | Other | Confidential, only for members of the consortium (including the Commission Services) | 2 |
| D3.2 | Standard assays, analytics and calculations for monitoring enzymatic performance | 4 - UHAM | Report | Confidential, only for members of the consortium (including the Commission Services) | 2 |
| D3.3 | Set of 100 best clones, 10 isolates, and 10 enzymes shortlisted for sequencing or transfer to WP2 | 6 - IST ID | Other | Confidential, only for members of the consortium (including the Commission Services) | 10 |
| D3.4 | Sequence, activity, and stability datasets from best positive bio-resources | 3 - BANGOR | data sets, microdata, etc | Confidential, only for members of the consortium (including the Commission Services) | 18 |
| D3.5 | Set of new bio-resources to screen or sequence | 7 - CNR | Other | Confidential, only for members of the consortium (including the Commission Services) | 24 |
| D3.6 | Complete set of positive naïve screened enzymes and sequences and their datasets | 3 - BANGOR | Report | Confidential, only for members of the consortium (including the Commission Services) | 32 |

# Milestones

| Milestone number[18] | Milestone title | Lead beneficiary | Due Date (in months) | Means of verification |
|---|---|---|---|---|
| MS9 | First round of functional screens completed | 6 - IST ID | 6 | Materials available – this milestone will attest the realisation of the first screens of available bio-resources. |
| MS10 | First round of sequencing completed | 3 - BANGOR | 6 | Data available – this milestone will attest to the realisation of the sequencing of the first selected bio-resources found to be positive in the screen tests. |
| MS11 | The first sampling campaign completed | 7 - CNR | 12 | Sites data, samples available - this milestone will attest completion of campaigns for sampling new bio-resources with information about sample sites available. |