ECC European Culture Collections' Organisation
XLII Meeting – Bari (IT) 18-20 September 2024

# Cold-adapted carboxylic ester hydrolases from two Antarctic *Psychrobacter* strains: genomic analyses and *in-vitro* studies

SUS-MIRRI.IT
PNRA19_00073 (TENORE)
PNRA18_00232 (AMICI)

Ca' Foscari University of Venice
Institute of Biomolecular Chemistry

**Andrea Cattaneo**
**PhD student in Polar Sciences**
**Institute of Biomolecular Chemistry ICB-CNR, Pozzuoli (IT)**

# OVERVIEW OF THE PhD PROJECT

**Sampling campaigns in Antarctica**

Environmental samples

**Genomic approach**

Study of bacterial communities

**Culturomic approach**

In-lab isolation of extremophilic bacteria

**Taxonomical characterization and study of biodiversity**

**Study of biomolecules**

**Polymers**

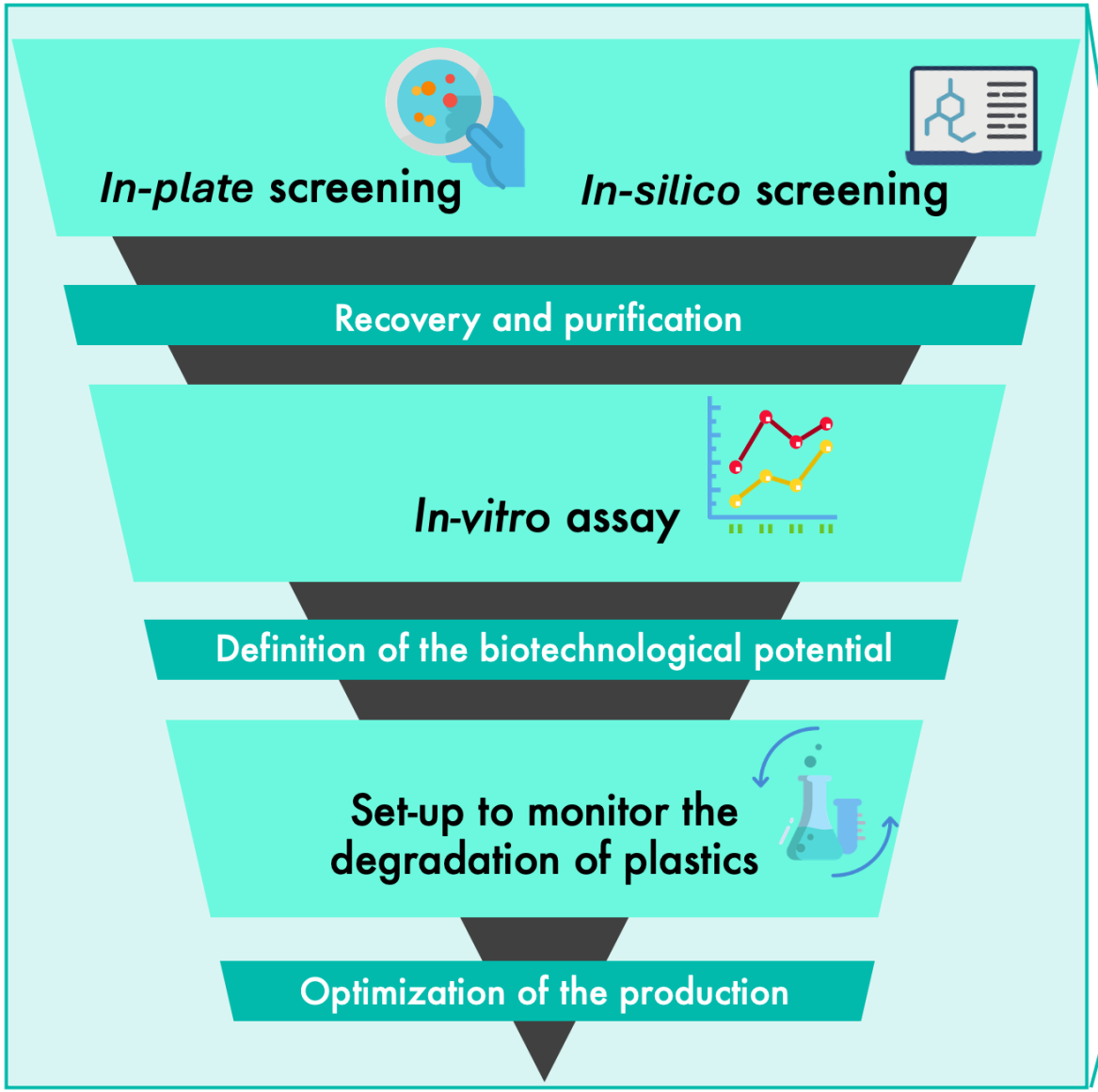
e.g. exo- and capsular-polysaccharides, polyglutamic acid

**Enzymes**

e.g. carboxylic ester hydrolases (EC 3.1.1), glycoside hydrolases (EC 3.2.1)

**Genome sequencing and annotation**

# OVERVIEW OF THE PhD PROJECT




Taxonomical characterization and study of biodiversity

Study of biomolecules

**Polymers**
e.g. exo- and capsular-polysaccharides, polyglutamic acid

**Enzymes**
e.g. **carboxylic ester hydrolases (EC 3.1.1)**, glycoside hydrolases (EC 3.2.1)

Genome sequencing and annotation

# ENVIRONMENTAL SAMPLING IN ANTARCTICA

## XXXIII Italian Antarctic expedition (2017/2018)
## XXXIV Italian Antarctic expedition (2018/2019)
**Supported by Research Project PNRA16_00274**

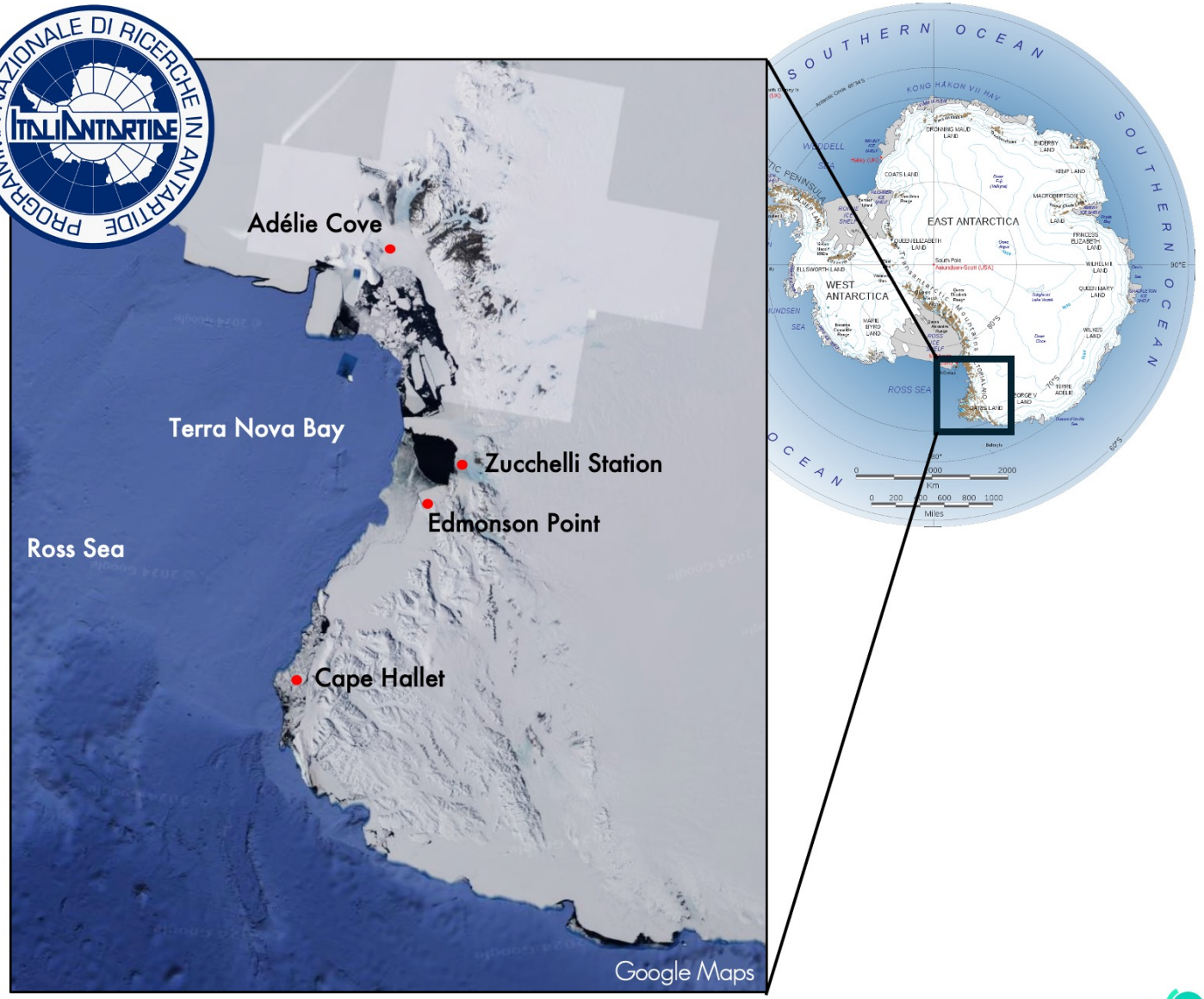
- Terra Nova Bay, in proximity of the Mario Zucchelli Station and the Adélie Cove Bay (ASPA 161)
  *Marine sediment samples from 20 and 50 m depth*

- Edmonson Point (ASPA 165)
  *Sand and sediment samples*

- Cape Hallet (ASPA 106)
  *Sand and sediment samples*

## XXXIX Italian Antarctic expedition (2023/2024)
**Supported by Research Project PNRA19_00073**

- Ross Sea polynya with Laura Bassi Research Vessel
  *Sea water samples*




Semprucci et al. (2021). Diversity. Antarctic Special Protected Area 161 as a Reference to Assess the Effects of Anthropogenic and Natural Impacts on Meiobenthic Assemblages.

# ENVIRONMENTAL SAMPLING IN ANTARCTICA

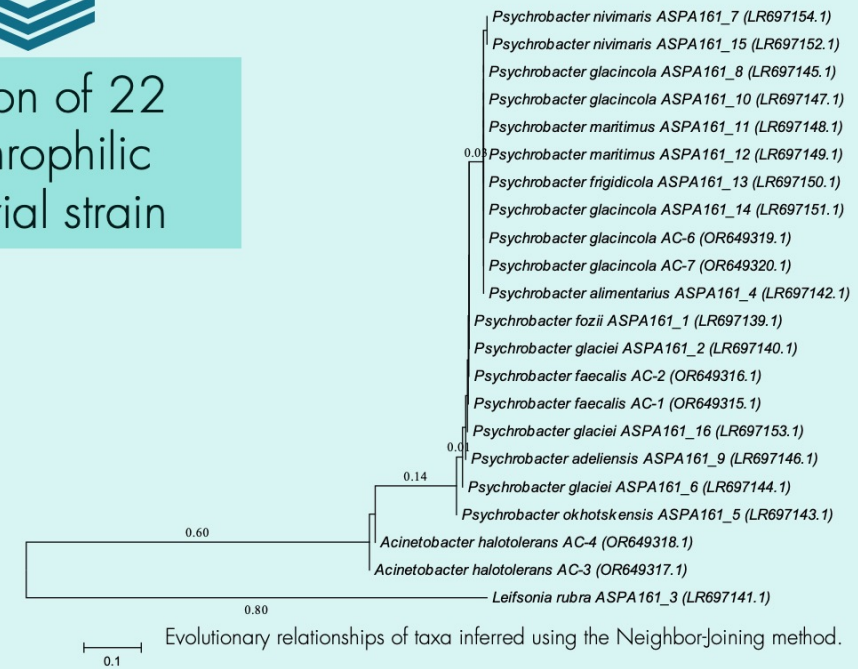
## XXXIII Italian Antarctic expedition (2017/2018)

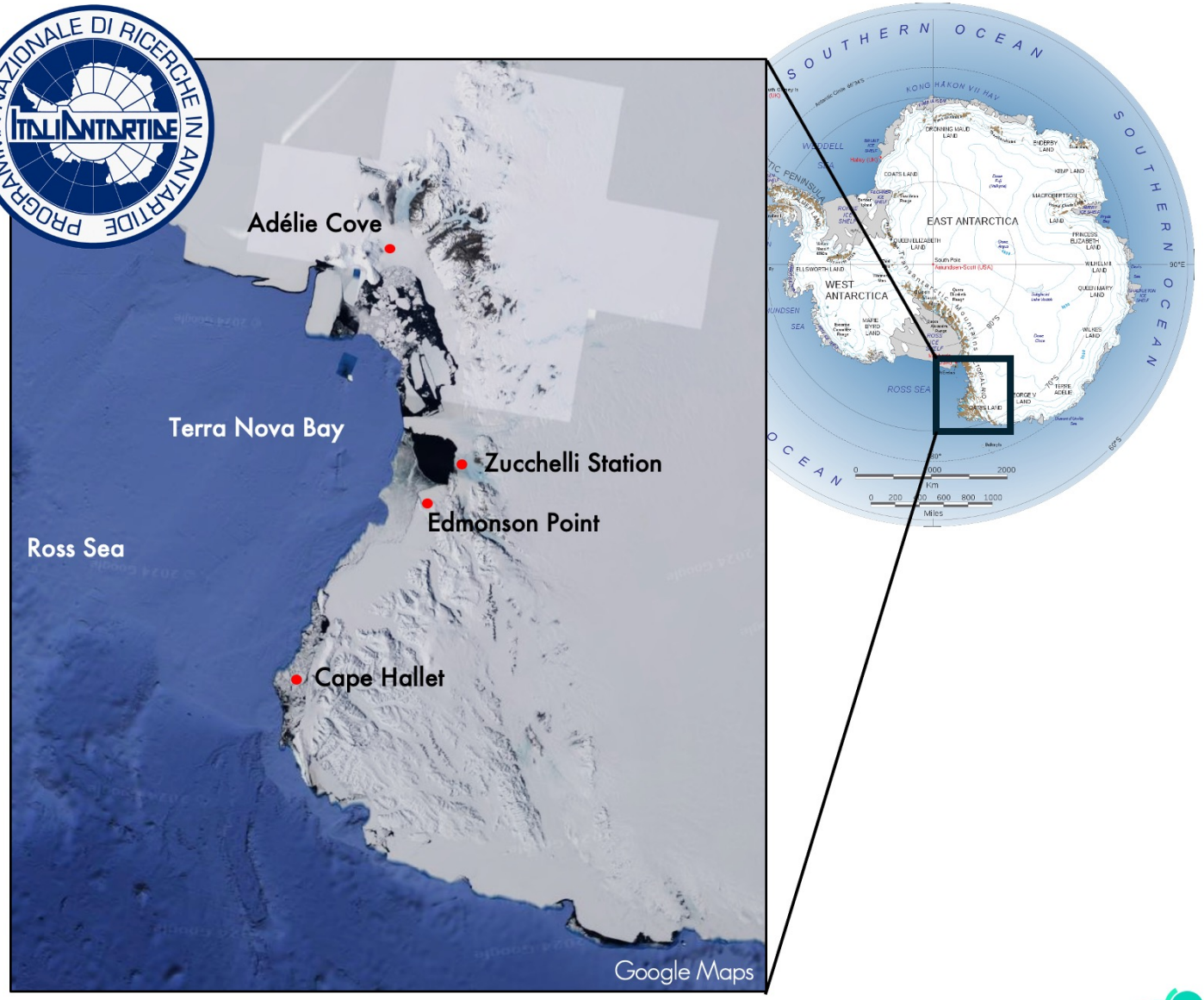
XXXIV Italian Antarctic expedition (2018/2019)

**Supported by Research Project PNRA16_00274**

- Terra Nova Bay, in proximity of the Mario Zucchelli Station and the Adélie Cove Bay (ASPA 161)
  *Marine sediment samples from 20 and 50 m depth*

Isolation of 22 psychrophilic bacterial strain




Semprucci et al. (2021). Diversity. Antarctic Special Protected Area 161 as a Reference to Assess the Effects of Anthropogenic and Natural Impacts on Meiobenthic Assemblages.

# STUDY OF EXTREMOPHILIC BACTERIA: ICB CULTURE COLLECTION

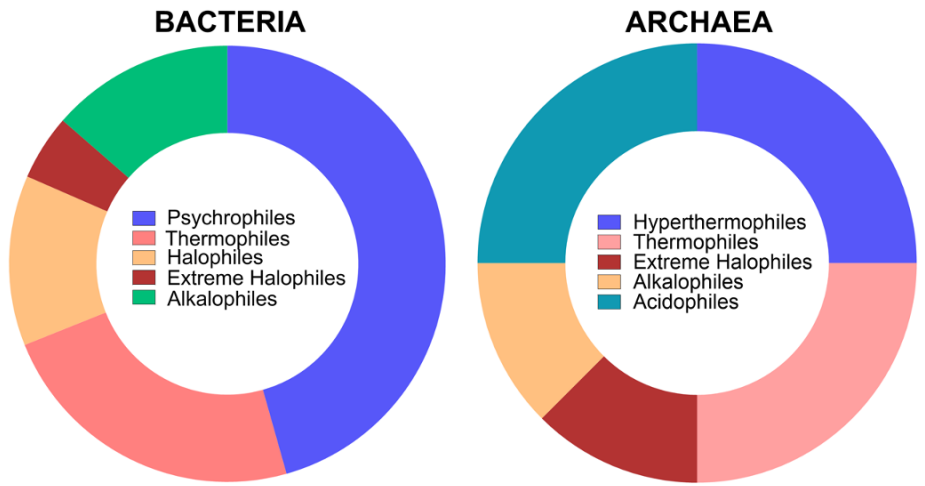
Culturomic approach

**CE-ICB Extremophiles Collection**
Institute of Biomolecular Chemistry – National Research Council (Napoli, IT)
Collection, characterization, distribution and preservation of microbial strains

**SUS-MIRRI.IT project**, funded by NextGeneration EU 2022

SUS-MIRRI.IT

More information @ our Posters




***Psychrobacter* sp. strain ASPA161_6**

***Psychrobacter* sp. strain ASPA161_9**

| Temperature | Medium | pH | Salinity |
|---|---|---|---|
| 10-30 °C, optimum 20 °C | Tryptic Soy Broth | 6.0-8.5 | 0.5-5% |
| 10-30 °C, optimum 20 °C | Tryptic Soy Broth | 6.0-9.0 | 0.5-5% |

# STUDY OF EXTREMOPHILIC BACTERIA: ICB CULTURE COLLECTION

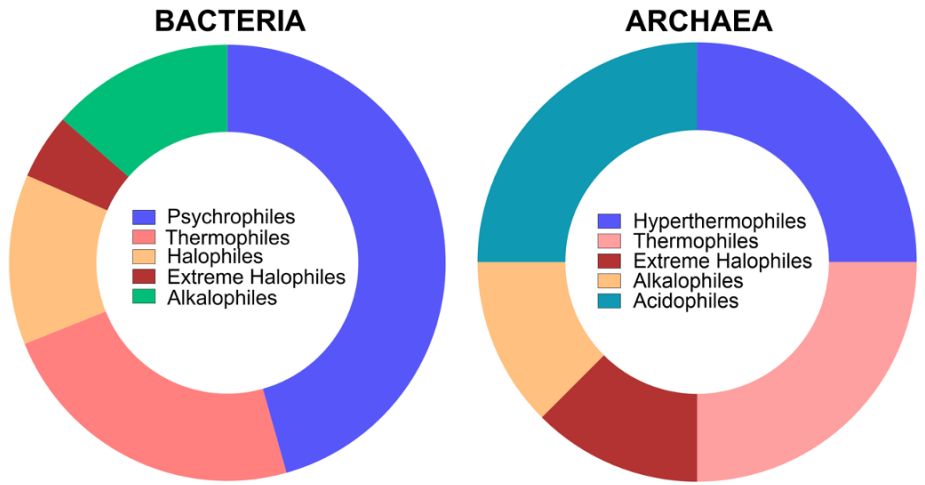
Culturomic approach

**CE-ICB Extremophiles Collection**
Institute of Biomolecular Chemistry – National Research Council (Napoli, IT)
Collection, characterization, distribution and preservation of microbial strains

**SUS-MIRRI.IT project**, funded by NextGeneration EU 2022

SUS-MIRRI.IT

More information @ our Posters




Genome sequencing: dDDH and ANI

**_Psychrobacter_ sp. strain ASPA161_6**

Accession number JBEFNJ000000000

| Genome compared | Access. number | dDDH [%confidence interval] | ANIb [%aligned nucleotide] | ANIm [%aligned nucleotide] |
|---|---|---|---|---|
| *Psychrobacter glaciei* KCTC 42280 | GCA_014652895 | 67.7 [64.8-70.6] | 95.72 [82.56] | 96.37 [84.12] |
| *Psychrobacter okhotskensis* MD17 | GCF_904846405 | 30.2 [27.8-32.7] | 85.06 [66.87] | 87.97 [60.20] |
| *Psychrobacter fjordensis* BSw21516B | GCF_904845995 | 25.7 [23.4-28.2] | 81.01 [59.89] | 86.97 [33.89] |
| *Psychrobacter fozii* CECT 5889 | GCA_003217155.1 | 25.5 [23.2-28.0] | 81.09 [60.76] | 86.82 [35.29] |

**_Psychrobacter_ sp. strain ASPA161_9**

Accession number JBEFNI000000000

| | | | | |
|---|---|---|---|---|
| *Psychrobacter okhotskensis* MD17 | GCF_904846405 | 28.8 [22.5-27.3] | 79.69 [55.73] | 86.29 [26.66] |
| *Psychrobacter fulvigenes* KC40 | GCF_904846155 | 24.3 [22.0-26.7] | 79.91 [57.12] | 85.63 [29.12] |
| *Psychrobacter maritimus* Pi2-20 | GCF_904846295 | 24.2 [21.9-26.7] | 79.44 [54.22] | 85.99 [24.65] |
| *Psychrobacter luti* CECT 5885 | GCF_014192115 | 23.9 [21.6-26.4] | 79.18 [52.68] | 85.74 [23.20] |

# *IN-SILICO* SCREENING

Genome annotation
Search for **cold-adaptation and stress responses** genes

| Gene | COG | Product | EC number |
|---|---|---|---|
| cspA_1 | COG1278 | Cold shock protein | / |
| cspA_2 | COG1278 | Cold shock protein | / |
| cspV | COG1278 | Cold shock protein | / |
| deaD | COG0513 | ATP-dependent RNA helicase | 3.6.4.13 |
| desA3 | COG3239 | NADPH-dependent stearoyl-CoA 9-desaturase | 1.14.19 |
| katE | COG0753 | Catalase C | 1.11.1.6 |
| katG | COG0336 | Catalase-peroxidase | 1.11.1.21 |
| sodA | COG0605 | Superoxide dismutase [Mn/Fe] | 1.15.1.1 |
| sodB | COG0605 | Superoxide dismutase [Fe] | 1.15.1.1 |
| msrAB | COG0225 | Peptide methionine sulfoxide reductase | 1.8.4.12 |

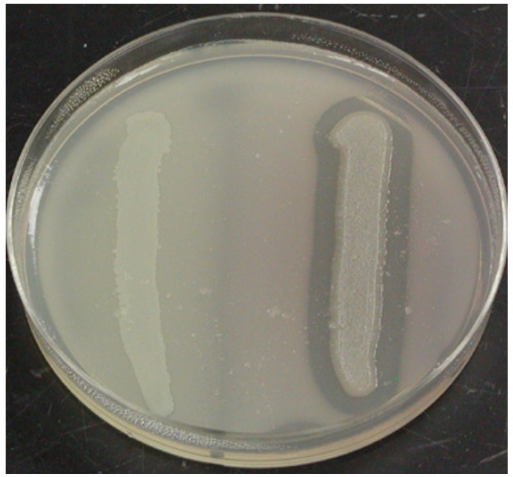
# *IN-SILICO* SCREENING

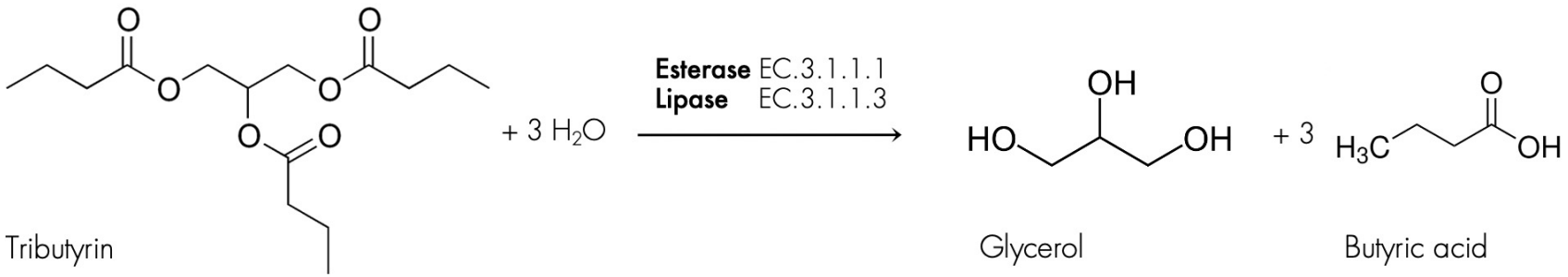
Genome annotation
Search for carboxylic ester hydrolases: **lipase (EC 3.1.1.3)** and **esterase (EC 3.1.1.1)**

| | Gene | Length (bp) | COG | Product | EC number |
|---|---|---|---|---|---|
| ***Psychrobacter* sp. strain ASPA161_6** | estB | 672 | COG0400 | Carboxylesterase 2 | 3.1.1.1 |
| | lip1 | 987 | COG1075 | Lipase 1 | 3.1.1.3 |
| | lip2 | 1302 | COG1075 | Lipase 2 | 3.1.1.3 |
| | lip3 | 945 | COG1075 | Lipase 3 | 3.1.1.3 |
| | lip | 1077 | COG1075 | Triacylglycerol lipase | 3.1.1.3 |
| ***Psychrobacter* sp. strain ASPA161_9** | estB | 672 | COG0400 | Carboxylesterase 2 | 3.1.1.1 |
| | lip2 | 1548 | COG1075 | Lipase 2 | 3.1.1.3 |
| | lip3 | 948 | COG1075 | Lipase 3 | 3.1.1.3 |
| | lip | 1053 | COG1075 | Triacylglycerol lipase | 3.1.1.3 |

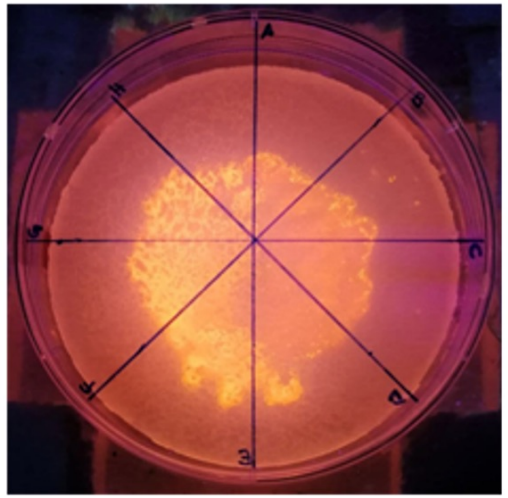
# *IN-PLATE* SCREENING FOR POLYESTER HYDROLASES

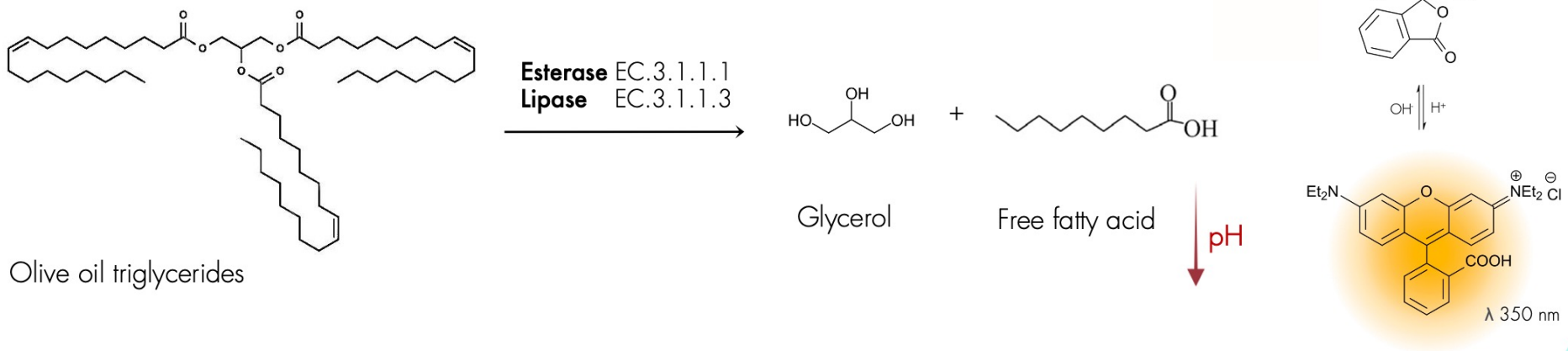
## Tributyrin 1% (v/v) agar plate






## Olive oil 2.5% (v/v) and rhodamine B agar plate



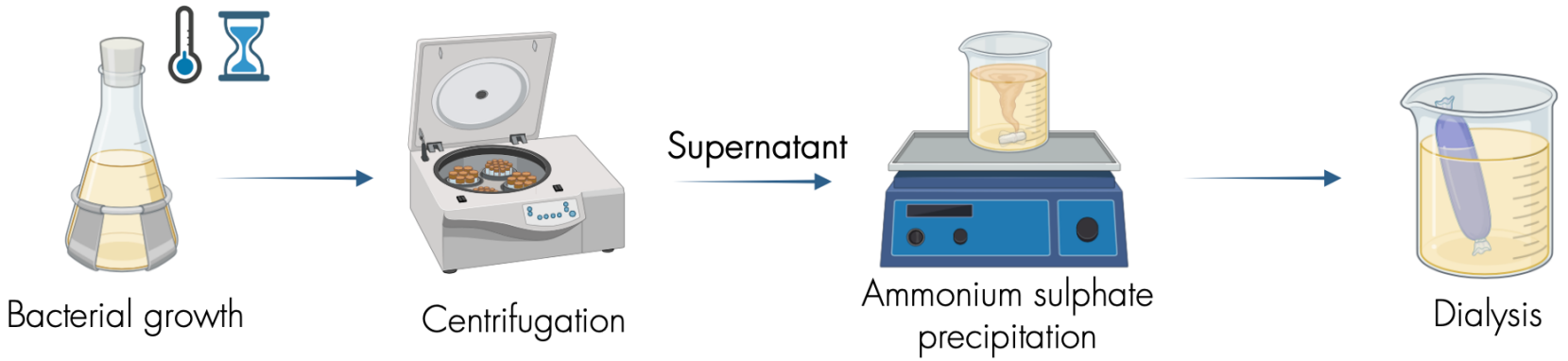




Wu and Tsai (2004). Enzyme Microb Technol. Kinetics of tributyrin hydrolysis by lipase.
Jaeger (1987). Appl Environ Microbiol. Specific and sensitive plate assay for bacterial lipases.

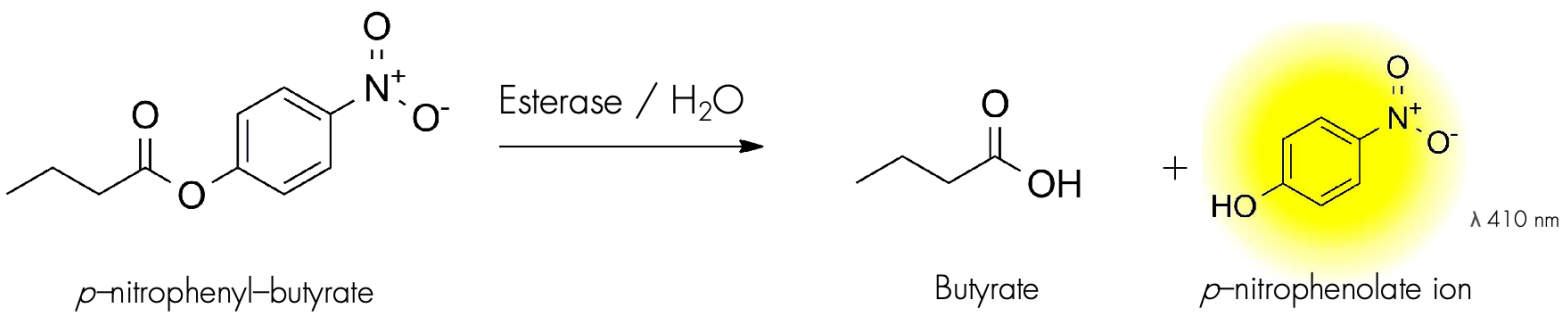
# *IN-VITRO* ASSAY: COLORIMETRIC-SPECTROPHOTOMETRIC ASSAYS

Recovery of the **extracellular enzymatic activities** in the partially purified supernatant




Created with Biorender.com

Colorimetric-spectrophotometric assays with chromogenic substrates: **p-NP esters**



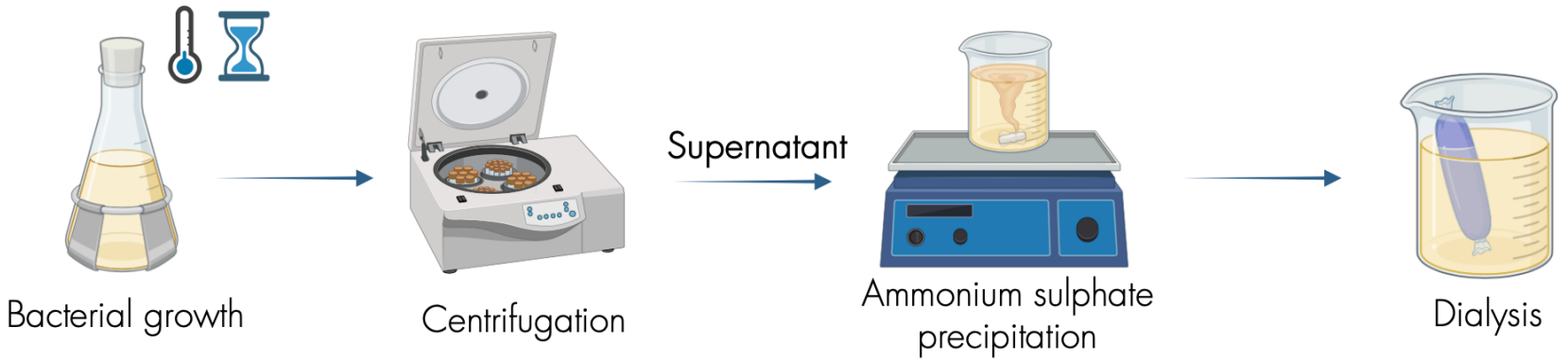

*p*–nitrophenyl–butyrate

Butyrate

*p*–nitrophenolate ion

# *IN-VITRO* ASSAY: COLORIMETRIC-SPECTROPHOTOMETRIC ASSAYS

Recovery of the **extracellular enzymatic activities** in the partially purified supernatant




Created with Biorender.com

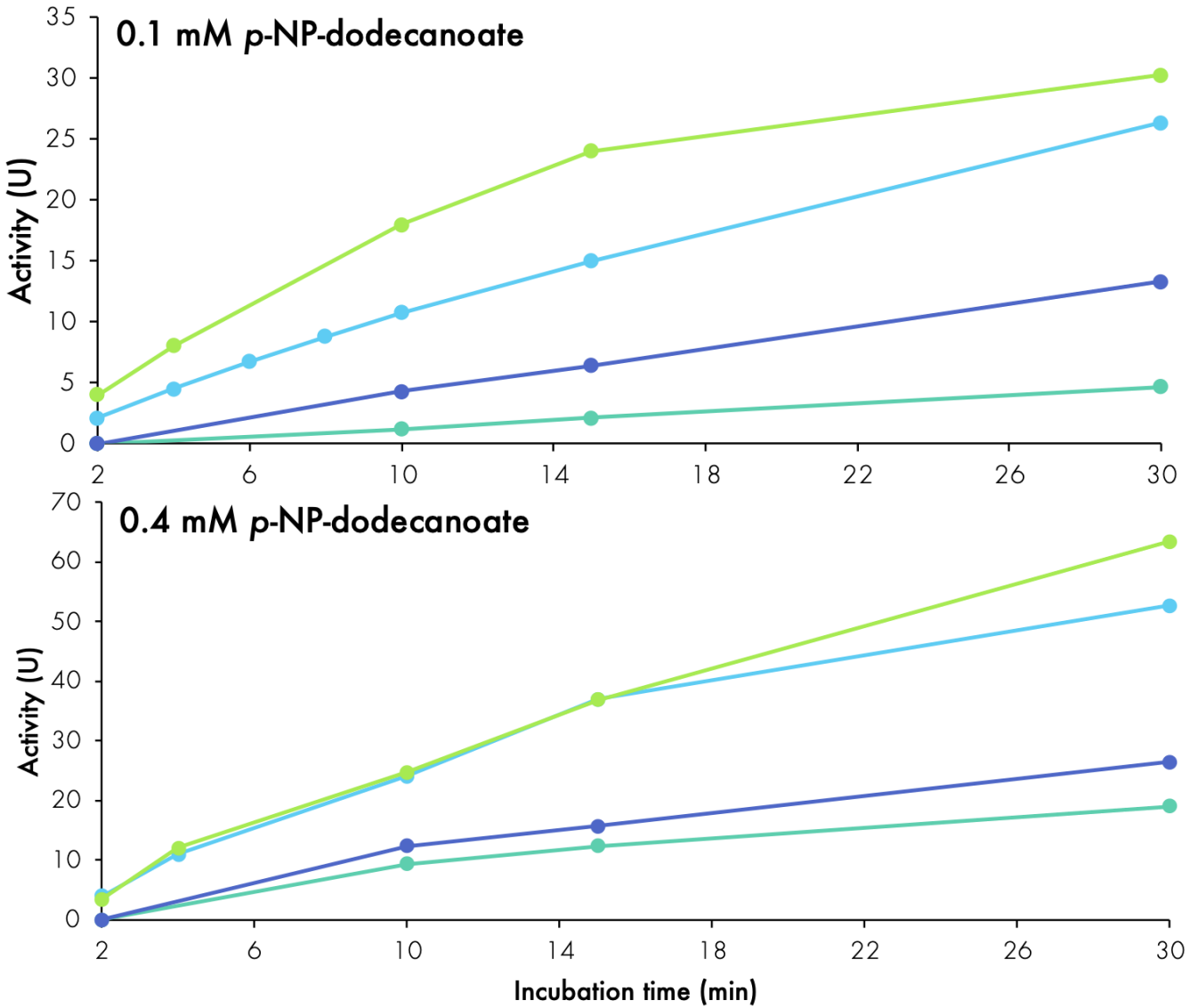
Colorimetric-spectrophotometric assays with chromogenic substrates: **p-NP esters**

| **Enzymatic activity** | **pNP esters** | **ASPA161_6** | **ASPA161_9** |
|---|---|---|---|
| **Esterase EC.3.1.1.1** | *p*–NP–acetate ($C_2$) | + | + |
| | *p*–NP–butyrate ($C_4$) | + | + |
| **Lipase EC.3.1.1.3** | *p*–NP–decanoate ($C_{10}$) | + | + |
| | *p*–NP–dodecanoate ($C_{12}$) | + | + |

# *IN-VITRO* ASSAY: COLORIMETRIC-SPECTROPHOTOMETRIC ASSAYS

Hydrolysis of ester bonds (static incubation, different temperatures and incubation times)

**EXAMPLE: *Psychrobacter* sp. strain ASPA161_9**

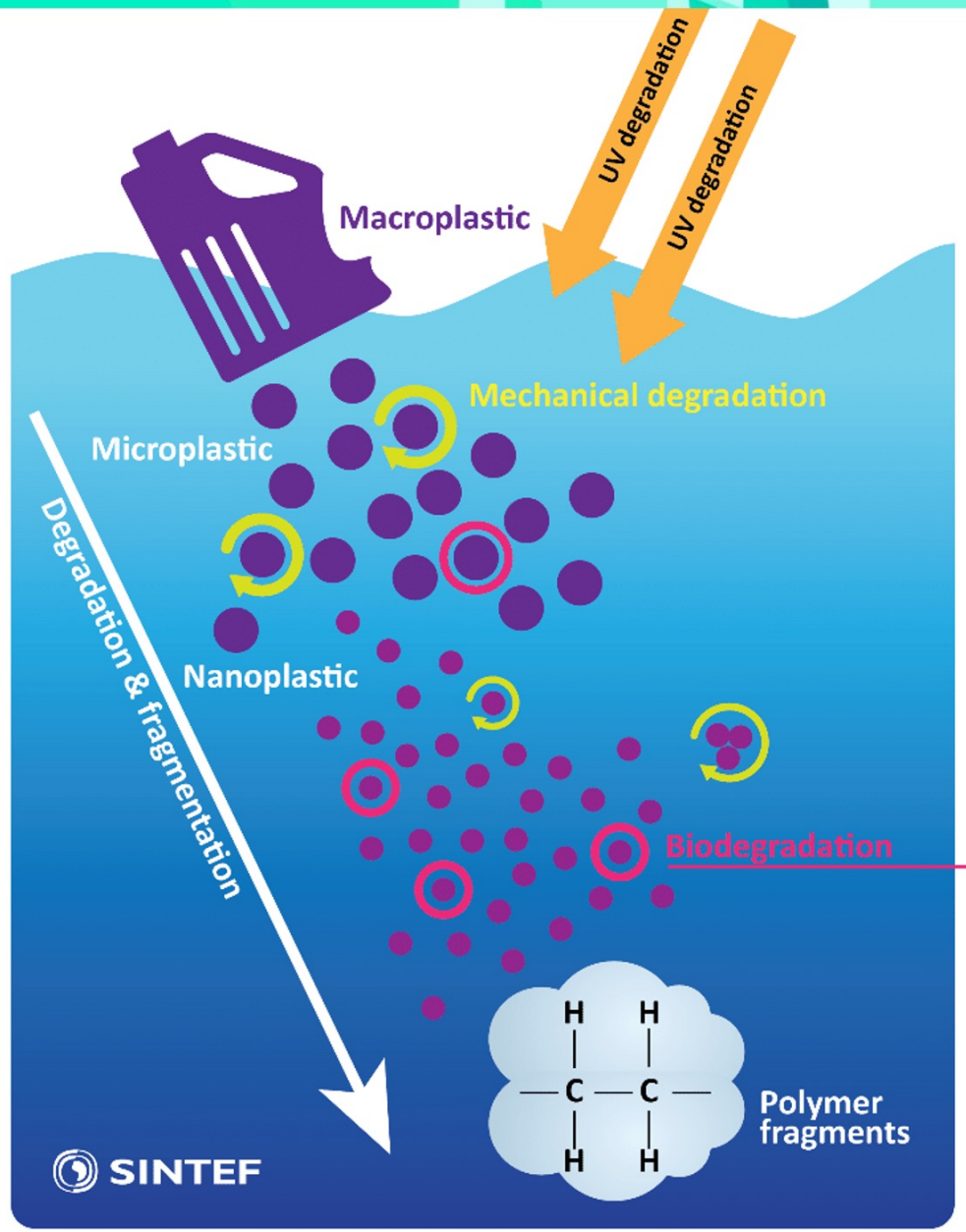


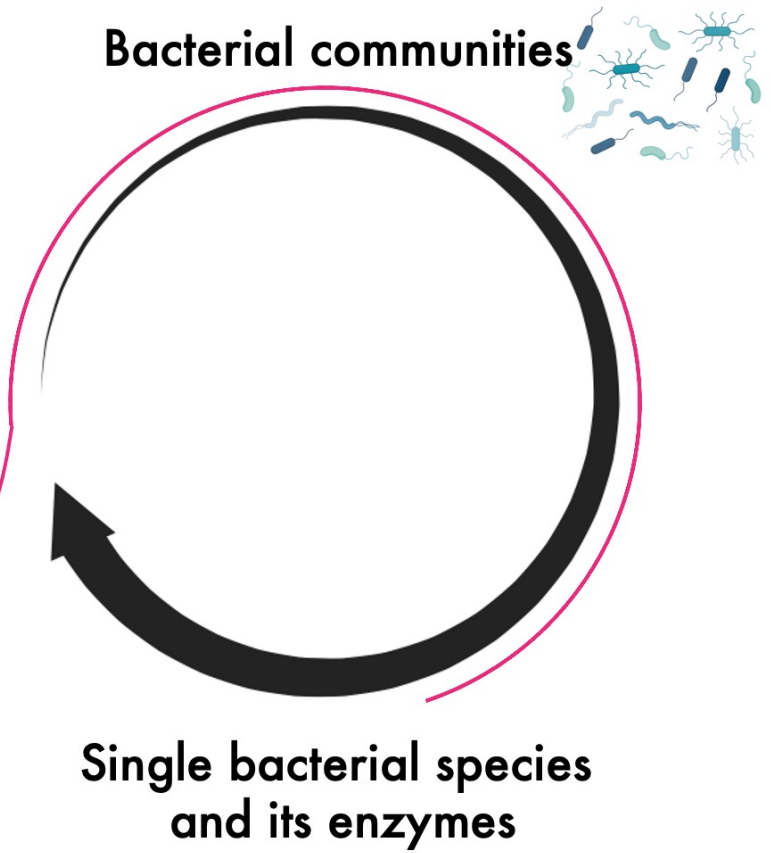

**Similar results with both strains and the different substrates**

- 35 °C
- 25 °C
- 15 °C
- 4 °C

1U: activity releasing 0.001 µmol of *p*-nitrophenol under the assay conditions

# INSIGHTS INTO PLASTIC POLLUTION AND MICROBIAL PLASTIC TRANSFORMATION

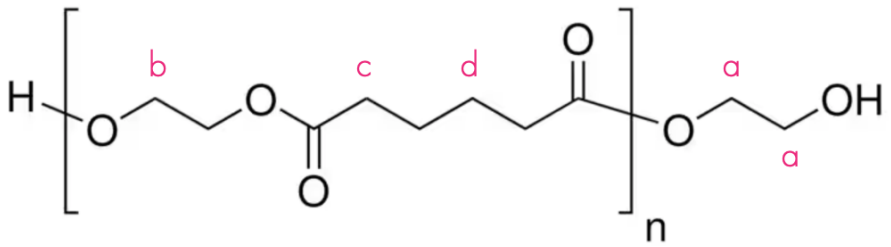


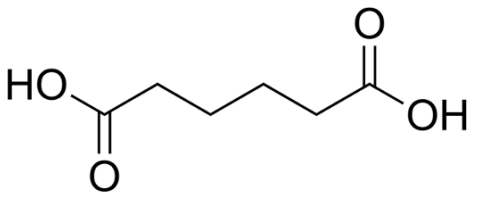

Booth et al. (2017). Microplastic in global and Norwegian marine environments: distributions, degradation mechanisms and transport

# SET-UP: CONTROL OF THE DEGRADATION OF PLASTICS

Poly(ethylene adipate) PEA

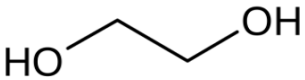


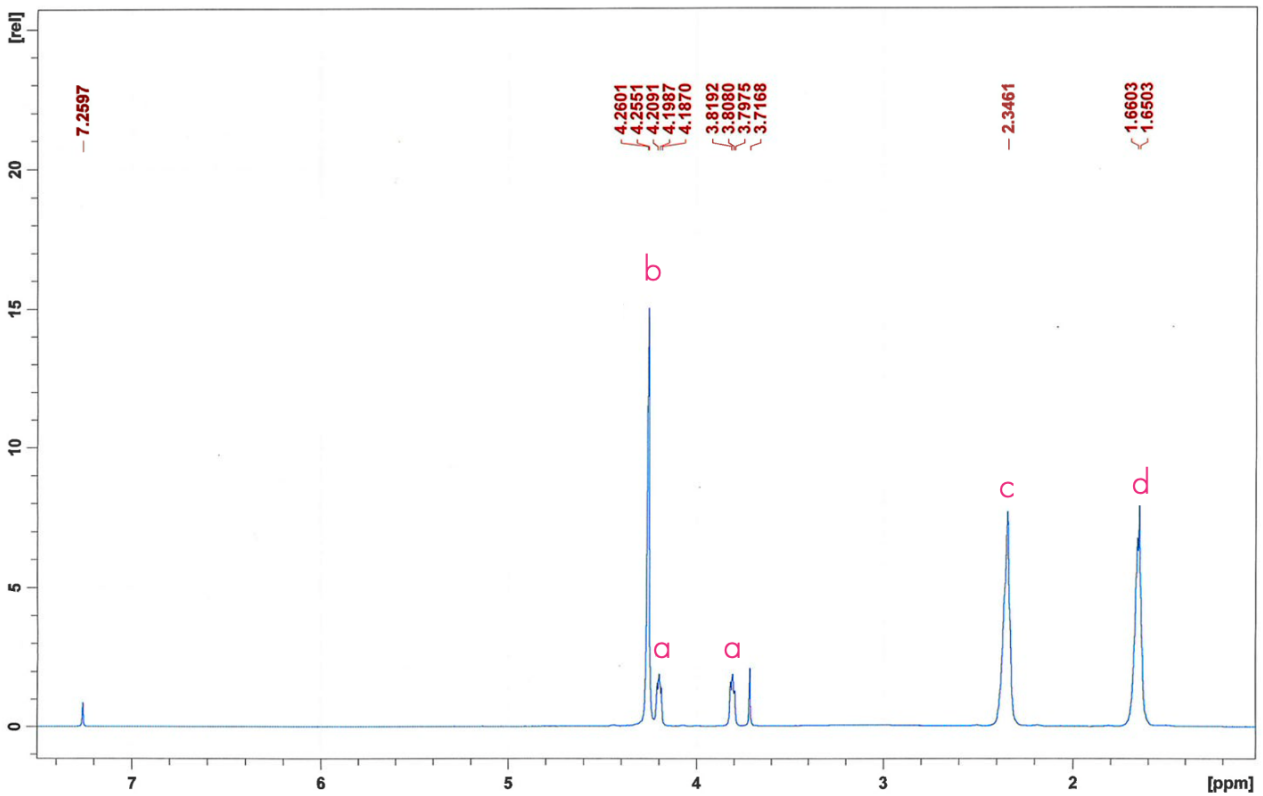

Adipic acid




\+

Ethylene glycol



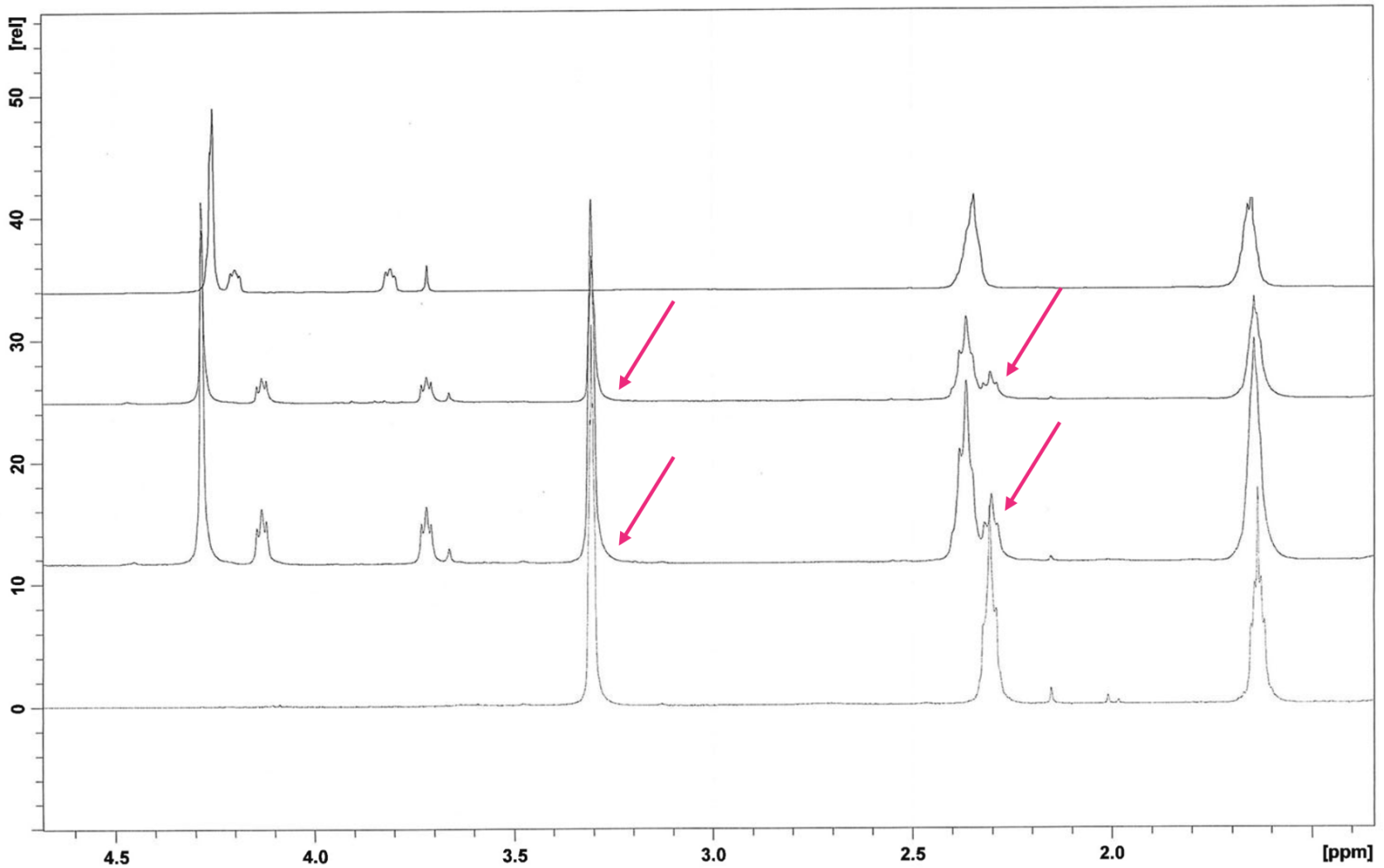

$^1$H NMR spectrum of PEA

Collaboration with C. Sanfilippo ICB-CNR Catania

Biodegradation time > 30 days (PEA film, 37 °C)

# SET-UP: CONTROL OF THE DEGRADATION OF PLASTICS

PEA degradation by **partially purified extracellular enzymes from the supernatant**




**31% degradation**
**7 days**

$^1$H NMR spectra

Collaboration with C. Sanfilippo ICB-CNR Catania

# SET-UP: CONTROL OF THE DEGRADATION OF PLASTICS

PEA degradation by **whole cells**

ON GOING

**Incubation 7 and 30 days**
**15 and 25 °C**

**NMR study**

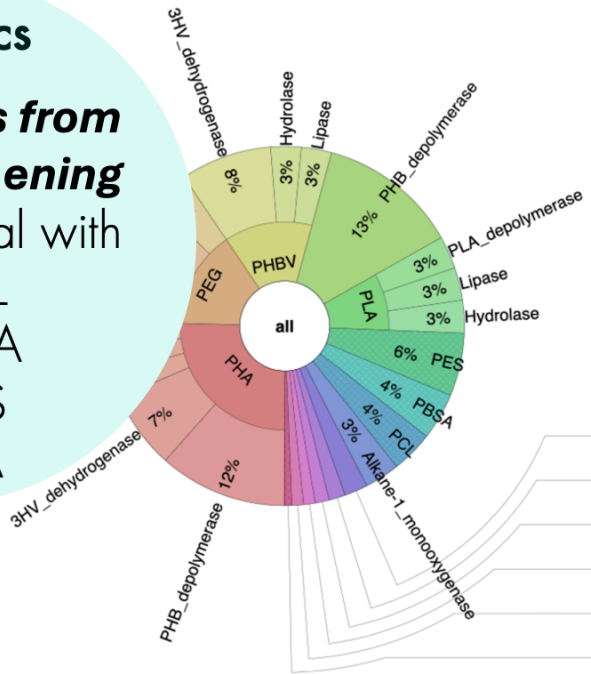
Collaboration with C. Sanfilippo ICB-CNR Catania

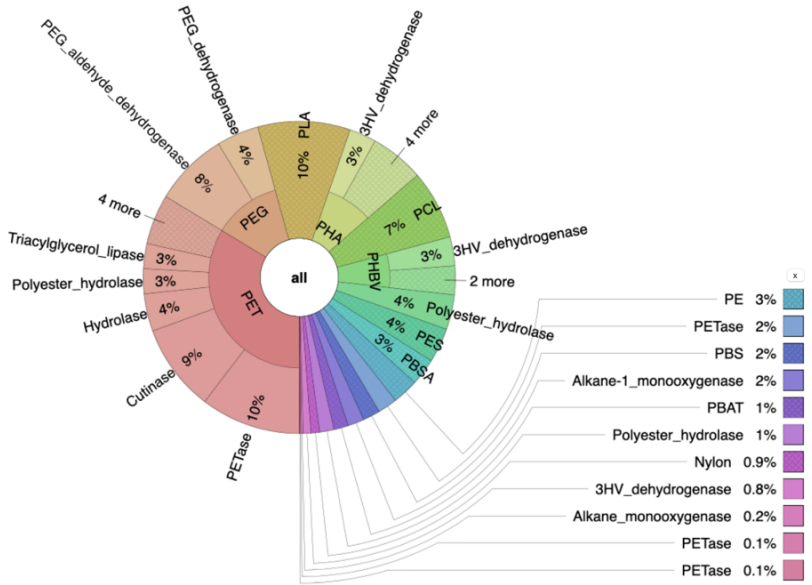
# NEXT STEPS

- **Improving** of the enzymatic degradation performances
- Setting-up of experiments for **bacterial cell-mediated degradation suitable with sea water temperature**
- **Gene cloning and expression** to produce enzymes of interest
- Evaluating **experiments onto other (bio)plastics**

***Results from in-silico screening***

potential with
- PCL
- PHA
- PES
- PLA




Collaboration with R. Papikyan, A.L.Takhtajan Institute of Botany, Yerevan (AM)

**Extremophilic Research Group**
ICB-CNR, Pozzuoli (IT)

Institute of Biomolecular Chemistry

**Annarita Poli** Research Manager
**Ilaria Finore** S5.5
**Ida Romano** S4.8
**Luigi Leone**
**Maria Vittoria** S4.5
**Antonia D'Amodio** S4.4
**Giovanni Pezzella** S4.6

**Collaborations**
**Claudia Sanfilippo** ICB-CNR, Catania (IT)
**Roberto Abbamondi** ICB-CNR, Pozzuoli (IT) S4.7
**Razmik Papikyan** A.L.Takhtajan Institute of Botany, Yerevan (AM)

**PhD Scientific Supervisors**
**Paola Di Donato** Parthenope University of Napoli (IT)
**Ilaria Finore** ICB-CNR, Pozzuoli (IT)
**Elena Chianese** Parthenope University of Napoli (IT)

SUS-MIRRI.IT

PNRA19_00073 (TENORE)
PNRA18_00232 (AMICI)

# Thank you for the attention!

ECCO | European Culture Collections' Organisation
XLII Meeting – Bari (IT) 18-20 September 2024