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*FuturEnzyme:*

Technologies of the Future for Low-Cost Enzymes for Environment-Friendly Products

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**SET OF 100 BEST CLONES, 10 ISOLATES,  
AND 10 ENZYMES SHORTLISTED FOR  
SEQUENCING OR TRANSFER TO WP2**  
D3.3

CARLA DE CARVALHO

IST-ID

Av. Rovisco Pais, 1049-001 Lisboa, Portugal

## Document information sheet

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<b>Authors:</b>	IST-ID (Carla de Carvalho, Patricia Gómez-Villegas), CSIC (Manuel Ferrer, Patricia Molina), Bangor (Anna Khusnutdinova, Peter Golyshin), UDUS (Fabienne Hilgers, Rebecka Molitor, Stephan Thies, Karl-Erich Jaeger), UHAM (Jennifer Chow, Wolfgang Streit), CNR (Mikhail Yakimov)
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<b>Contact details:</b>	Carla de Carvalho (ccarvalho@tecnico.ulisboa.pt), Manuel Ferrer (mferrer@icp.csic.es)

Summary

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# SET OF 100 BEST CLONES, 10 ISOLATES, AND 10 ENZYMES SHORTLISTED FOR SEQUENCING OR TRANSFER TO WP2

## 1. Scope of deliverable

This deliverable aimed at selecting a set of bio-resources that represent best candidates for the purposes of FuturEnzyme's objectives as they match the requirements of the industrial partners summarized in Deliverable 2.1. Such candidates deserved further analysis, including experimental analysis and genome or metagenome sequencing. Some of these bio-resources were generated in the framework of previous European and national-funded projects, and were compiled and exchanged within the consortium at the beginning of the project for screening those relevant to FuturEnzyme (see Deliverable D3.1), whereas others were newly generated during the project (see Deliverable D3.4). A set of at least 10 single enzymes, 100 clones representing DNA fragments of microbial communities, and 10 microbial isolates containing enzymes, for the purposes of FuturEnzyme's objectives, were the initial objective (according to the initial proposal). From the total bio-resources available, that included the one generated in the framework of previous European and national-funded projects and the newly generated during the project, 257 new enzymes (retrieved after sequencing of metagenomics clones, or after re-screening available enzymes) and 41 isolates with interesting activities have been pre-selected. Currently, the genomes of 22 isolates and the metagenomes of 54 environmental samples were selected for sequencing and their sequences have been made available within the consortium. The list of bio-resources and the QR code is available in the internal FuturEnzyme repository, and also as a report that will accompany this deliverable (the present document).

## 2. Reasons for the update

The first version of the Deliverable D3.3 was submitted in March 2022. This update is due to the fact that since the submission, the partners were able to pre-select a new set of bio-resources for experimental and sequencing focus. This was a consequence of the partners efforts to collect a larger number of bio-resources that were available but not catalogued, and newly generated samples, as it is extensively described in the Deliverable D3.1 "Bio-resources prepared and exchanged" and D3.4 "Bioresources new for FuturEnzyme". In November 2022, the Coordinator (Manuel Ferrer) contacted the Project Officer (Colombe Warin) to explain these circumstances and ask her to re-open the submission of this deliverable (amongst others), at which she agreed.

## 3. Origin of the deliverable

Along the already 18 months of project, two deliverables have been accomplished from which the present one nourishes. To be mentioned:

- D3.1: Bio-resources prepared and exchanged (July, 2021, updated December 2022)  
*This deliverable enlists a set of bio-resources (enzymes, isolates, enrichment cultures, clone metagenomic libraries, genomes and shotgun metagenome sequences) available within the consortium. They represent bio-resources generated in the framework of previous European and national-funded projects, and that were compiled and exchanged within the consortium, at the beginning of the project, for screening those relevant to FuturEnzyme.*
- D3.4: Sequence, activity, and stability datasets from best positive bioresources (November 2022)  
*This deliverable consists in the datasets informing about the sequences, performances and stabilities of best preselected bio-resources (isolates and clones), as well the information of bio-resources newly generated during the project.*

## 4. Methodology and Results

The starting point of this deliverable is multiple:

- On one hand, the bio-resources generated in the framework of previous European and national-funded projects, and that were compiled and exchanged within the consortium, at the beginning of the project, for screening those relevant to FuturEnzyme (Deliverable D3.1). It is worth noting that these bio-resources were not screened at the start date of the project, for the purposes of FuturEnzyme's objectives. They include the following:
  - Enzymes: 1428
  - Isolates: 1482, of which 223 have their genomes accessible
  - Clone metagenomic libraries: 35
  - Enrichment cultures: 52
  - Shotgun metagenome sequences: 64
- On the other hand, the bio-resources newly generated during the project (Deliverable D3.4)
  - Raw environmental samples: 51 new samples.
  - Isolates: 208 new isolates from 28 different locations.
  - Clone metagenomic libraries: 7 fosmid libraries, altogether gathering libraries of at least 2 million clones.
  - Enrichment cultures: 42 enrichment cultures.
  - Shotgun metagenome sequences: 54 shotgun metagenomes.

By applying the protocols extensively described in the Deliverable D3.2 "Standard assays, analytics and calculations for monitoring enzymatic performance" (July 2021, updated December 2022) to all above bio-resources, the following candidates did show interesting features:

- A total of 257 new enzymes with interest, that have been disclosed at this point of the project:
  - 185 enzymes with esterase, lipase, or polyester degrading hydrolase activities
  - 70 enzymes with glycosyl hydrolase activity, 30 being hyaluronidases
  - 2 enzymes with proteolytic activity
- A total of 41 isolates with interesting activity for the project (from 18 different sites):
  - 19 being positive for esterase or lipase activity
  - 20 being positive for glycosyl hydrolase activity, 17 with hyaluronidase activity
  - 1 being positive for protease activity

In addition to that, the genomes of 22 isolates and the metagenomes of 54 environmental samples were selected for sequencing and their sequences have been made available within the consortium and transferred to WP2.

The ID name, the screening method by which each enzyme, clone, isolates or metagenome were pre-selected and the identity further retrieved, the enzyme class, the origin and the nature of the bio-resource and the corresponding enzyme, the amino acid, genome or metagenome sequences, and the GPS coordinates (if available), have been extensively detailed in Deliverables D3.1 "Bio-resources prepared and exchanged" (November 2022) and D3.4 "Sequence, activity, and stability datasets from best positive bioresources" (November 2022), and for this reason they are not repeated in this deliverable. For details see Deliverables D3.1 and D3.4, and Annex Tables 1 and 2.

## Annex 1\_D3.3

**Table 1.** Detailed information of bio-resources available at month 2. The different types of bio-resources are: enzymes, metagenomic clone libraries, enrichment cultures, shotgun metagenomes, isolates (including those with genomes sequenced). The table is available under the designation *Table D3.1\_Bioresources\_Month 2\_30.11.2022* at the FuturEnzyme web intranet through the following QR code (password needed), in the section *Shared data, Datasets*:



**Table 2.** Detailed information of bio-resources newly sampled and generated since the beginning of the project. The different types of bio-resources are: new samples, new metagenomic clone libraries, new enrichment cultures, new shotgun metagenomes, and new isolates (including those with genomes sequenced). The table also contains information of the series of enzymes and microorganisms with activities of interest to the project identified by performing functional tests of the bio-resources available at month 2 (**Table 1**), and newly generated during the project (**Table 2**). The table is available under the designation *Table D3.4\_Bioresources\_New for FuturEnzyme\_30.11.2022*) at the FuturEnzyme web intranet through the following QR code (password needed), in the section *Shared data, Datasets*:

