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

D3.3

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Document information sheet

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Summary

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 single enzymes (at least 10), as well as clones representing DNA fragments of microbial communities (at least 100) and microbial isolates (at least 10) containing enzymes, that represent best candidates matching the requirements of the industrial partners summarized in Deliverable 3.1; they will cover all nine enzymes classes to be targeted in the project. Their identities and associated QR codes are listed, and datasets informing about their performances and stabilities are also available via QR code, and will be updated continuously during the project life time and further.

The datasets available through QR codes in this report, detail the origin, identity and culture conditions of the microbes producing each enzyme (hosts, isolates), as well as the cloning system, the host, the vector, the inductor, the antibiotic resistance, etc. It also contains information regarding the origin of the sample (GPS coordinates, microbe or microbial community, environmental conditions, etc.), the laboratory and person responsible for the sampling and further processing steps.

The list of selected clones containing DNA-fragments, isolates and enzymes, and their respective QR codes, will be available for all partners in the internal FuturEnzyme repository.

2. Introduction

In the frame of *WP3_Activity-based bio-prospecting for enzymes,* along these first 10 months of project, testing of activities has been a priority in order to have as much candidates as possible for *WP2_Machine learning enzyme bio-prospecting integrated into an industrial context*. The bio-prospecting has been performed through bio-resources from previous FP7, H2020 and EraNet funded projects of the partners involved (from at least 420 geographically and environmental diverse extreme and non-extreme environments around the world, detailed in *D3.1 Bio-resources prepared and exchanged*), as well as databases search. To quest for prospects for the applications of interest for FuturEnzyme, the protocols detailed in deliverable *D3.2_Standard assays, analytics and calculations for monitoring enzymatic performance* have been followed by partners. It is worth noting that when mentioning clones, we were referring to fosmids or cosmids representing DNA fragments of microbial communities that were obtained from a metagenomic sample. Activity is tested from those clones and the gene codifying the enzyme responsible for such activity is identified after sequencing. Since finally we have an enzyme, we have included all in this category.

At the moment, the partners implicated in this deliverable (IST-ID, CSIC, Bangor, UDUS, UHAM, CNR) have identified a total of 155 promising candidates to be transferred to WP2. These prospects have been pointed out and will be further characterised for their potential applications detailed in *D2.1_Manufacturers' needs and specifications, protocol*. In order to fulfil FAIR and RRI purposes, all of them have a QR code associated which leads to the dataset compiling all the information regarding the enzyme/isolate, including the person responsible for the material/results (based on the template specifically prepared for the project, circulated to the partners and available in the private area of FuturEnzyme's website).

In the present document, a brief of the results is presented, and QR codes directing to the full lists (protected with password) with more details are given below. In this list documents, every entry corresponding to an enzyme/isolate is associated to a QR code driving to the mentioned dataset where all the information available is given. These datasets will be updated whenever new information is obtained within the same QR code. These documents are accessible in the private area of the FuturEnzyme's website.

3. Isolates/microorganisms

The FuturEnzyme partners have selected 22 isolates corresponding to microorganisms assigned to Archaea and Bacteria, showing activities of interest for our project (**Table 1**). Amongst them, the activities detected are oxidoreductases (EC1), and hydrolases including hyaluronidases (EC3.2.1.35, 3.2.1.36, 4.2.2.1), amylases (EC3.2.1.1), proteases (EC3.4.21), esterases (EC3.1.1.1), lipases (EC3.1.1.3) and cutinases (EC3.1.1.74). Some isolates show performance with different types of substrates, for instance *(Halo)-Pseudomonas aestusnigri*, which displays lipase, esterase, cutinase and oxidoreductase activities.

				Number of isolates with activity/ies	First priority for (industrial partner/s)	Second priority for (industrial partner/s)
EC 1	Oxidoreductase			3	Schoeller	Henkel
EC 3	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	

Table 1. Activities of the isolates selected as candidates for further analysis and the industrial partners for which they are of interest.

The following QR code directs to the full list of candidate isolates (password: FuturEnzyme€01/06/2021).

4. Enzymes

The FuturEnzyme partners have selected 133 enzymes as the most promising for the purposes of our project (**Table 2**). Amongst them, the activities detected are oxidoreductases (laccases, EC1.10.3.2), and hydrolases including hyaluronidases (EC3.2.1.35, 3.2.1.36, 4.2.2.1), amylases (EC3.2.1.1), cellulases (EC3.2.1.4), proteases (EC3.4.21), esterases (EC3.1.1.1), lipases (EC3.1.1.3) and poly-ester hydrolases (EC3.1.1.-).

				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	35	Henkel/ Schoeller	
			Lipase	3	Henkel/ Schoeller	
			Poly-ester hydrolase	89	Schoeller	Henkel

Table 2. Activities of the enzymes selected as candidates for further analysis and the industrial partners for which they are of interest.

The following QR code directs to the full list of candidate enzymes (password: FuturEnzyme€01/06/2021).

5. Conclusions and next steps

The 155 prospects (up to 163, 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). They are distributed amongst all the classes proposed (**Figure 1**) with the exception of PluriZymes which will be engineered in coming tasks (*Task 5.2_ Developing disruptive PluriZymes with multipurpose activities, D5.2_ Set of 18 mutants generated by genetic engineering*). Because of their outstanding capacities for the objectives of FuturEnzyme, they will be transferred to WP2 for homology/computational screen to feed the predictive tool and WP4 for enzyme characterisation for selecting those with manufacturers' specifications (Task 4.4).
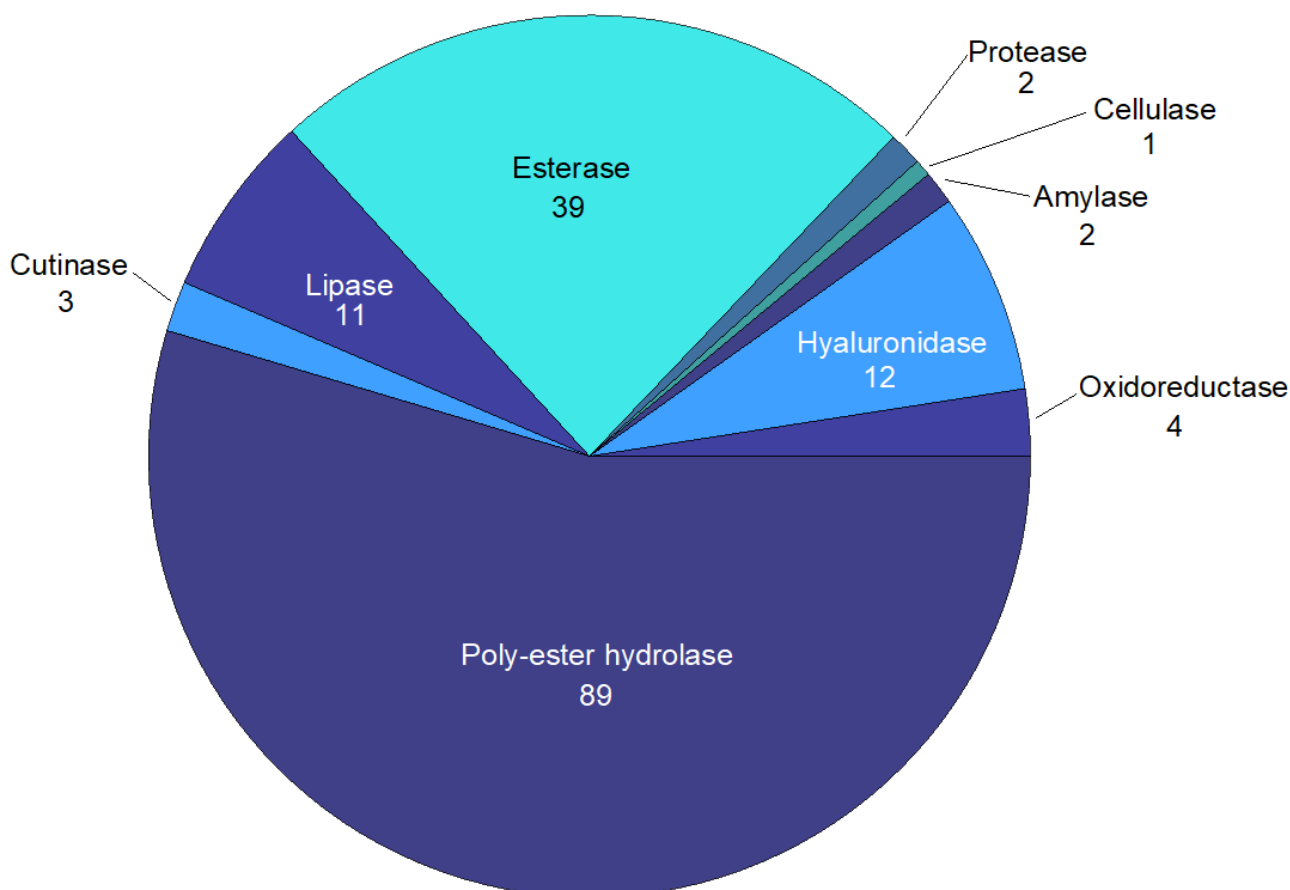

Figure 1. Distribution by activity of the enzymes and microorganisms selected in this deliverable.