

*Horizon 2020 Work programme*

Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research and the Bioeconomy

*Call*

H2020-FNR-2020: Food and Natural Resources

*Topic name*

FNR-16-2020: ENZYMES FOR MORE ENVIRONMENT-FRIENDLY CONSUMER PRODUCTS

*FuturEnzyme:*

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

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First set of 250 enzymes expressed at mg scale

MS12

## Document information sheet

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Summary

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## FIRST SET OF 250 ENZYMES EXPRESSED AT MG SCALE

## 1. Introduction

In the frame of *WP2\_Machine learning enzyme bio-prospecting integrated into an industrial context* and *WP3\_Activity-based bio-prospecting for enzymes*, this milestone attests the realisation of the first production batches for 250 enzymes (report/material available).

## 2. Bio-prospecting and production

To search for the best candidates (which are detailed in deliverable *D3.3\_Set of 100 best clones, 10 isolates, and 10 enzymes shortlisted for sequencing or transfer to WP2*), the analysis of 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)* and databases search were performed.

From all those selected sequences, 227 enzymes were produced in an appropriate heterologous expression organism at mg scale (for more details, go to QR codes in D3.3, available in the private area of FuturEnzyme’s website) and tested. Moreover, protein material from 22 isolates were also screened for interesting activities. For that purpose, the activity assays employed by the partners involved (IST-ID, CSIC, Bangor, UHAM, UDUS, and CNR) were those standardised in deliverable *D3.2\_Standard assays, analytics and calculations for monitoring enzymatic performance.* Figure 1 shows the different types of activities covered by the enzymes and isolates successfully expressed at milligram scales.



**Figure 1**. Distribution by activity of the enzymes and microorganisms described in this milestone.

## 3. List of enzymes and isolates

|  | ID | Activity | Type of material | To D3.3 |
| --- | --- | --- | --- | --- |
| 1 | Abo\_Est3 | Esterase | Enzyme | Yes |
| 2 | Abo\_LipD | Esterase | Enzyme | Yes |
| 3 | Dim008 | Esterase | Enzyme | Yes |
| 4 | Paes\_Est3 | Esterase | Enzyme | Yes |
| 5 | Paes\_PE-H\_Y250S | Esterase | Enzyme | Yes |
| 6 | Paes\_PE-H | Esterase | Enzyme | Yes |
| 7 | pelB::Paes\_TB035 | Esterase | Enzyme | Yes |
| 8 | PtEst1 | Esterase | Enzyme | Yes |
| 9 | TBEc304 | Esterase | Enzyme | Yes |
| 10 | TBEc310 | Esterase | Enzyme | Yes |
| 11 | BSU0630 | Laccase, Cu-oxidase | Enzyme | Yes |
| 12 | gen0081 | G13, α-amylase | Enzyme | Yes |
| 13 | gen0084 | Glycosyl hydrolase, GH3, cellulase | Enzyme | Yes |
| 14 | FE\_Polur1 | Esterase | Enzyme | Yes |
| 15 | FE\_Lip5-S42 | Esterase | Enzyme | Yes |
| 16 | FE\_Lip5-S46 | Esterase | Enzyme | Yes |
| 17 | Lip9-S42 | Esterase | Enzyme | Yes |
| 18 | Lip9-S46 | Esterase | Enzyme | Yes |
| 19 | EH0 | Esterase | Enzyme | Yes |
| 20 | EH1A1 | Esterase | Enzyme | Yes |
| 21 | EH3 | Esterase | Enzyme | Yes |
| 22 | EH7 | Esterase | Enzyme | Yes |
| 23 | EH11 | Esterase | Enzyme | Yes |
| 24 | EH15 - EstA1 | Esterase | Enzyme | Yes |
| 25 | EH26 | Esterase | Enzyme | Yes |
| 26 | EstA8 | Esterase | Enzyme | Yes |
| 27 | EH20 - EstA2 | Esterase | Enzyme | Yes |
| 28 | EH29 - EstA5 | Esterase | Enzyme | Yes |
| 29 | EH30 - EstB2 | Esterase | Enzyme | Yes |
| 30 | EH22 – EstA6 | Esterase | Enzyme | Yes |
| 31 | EH21 | Esterase | Enzyme | Yes |
| 32 | Meth3 | Esterase | Enzyme | Yes |
| 33 | EH37 | Esterase | Enzyme | Yes |
| 34 | EH37\_36E | Esterase | Enzyme | Yes |
| 35 | EH37\_87G | Esterase | Enzyme | Yes |
| 36 | EH37\_242G | Esterase | Enzyme | Yes |
| 37 | IS12 | Esterase | Enzyme | Yes |
| 38 | EH73 | Esterase | Enzyme | Yes |
| 39 | HRDSV\_2334 | Hyaluronidase | Enzyme | Yes |
| 40 | Fem04\_1 | Protease | Enzyme | Yes |
| 41 | D2-1 | Lipase | Enzyme | Yes |
| 42 | D2-2 | Lipase | Enzyme | Yes |
| 43 | I3-1 | Lipase | Enzyme | Yes |
| 44 | 1-1\_lipase\_10c\_11 | Hyaluronidase | Enzyme | Yes |
| 45 | 3-1\_esterase\_1c\_18 | Hydrolase | Enzyme | Yes |
| 46 | 5-1\_esterase\_13c\_9 | Hydrolase | Enzyme | Yes |
| 47 | 7-1\_esterase\_30c\_5 | Hydrolase | Enzyme | Yes |
| 48 | 8-1\_αβhydrolase\_14c\_19 | Hydrolase | Enzyme | Yes |
| 49 | 11-1\_αβhydrolase\_33c\_4 | Hydrolase | Enzyme | Yes |
| 50 | 12-1\_αβhydrolase\_43c\_3 | Hydrolase | Enzyme | Yes |
| 51 | 14-2\_esterase\_26c\_4 | Hydrolase | Enzyme | Yes |
| 52 | 15- 2\_esterase\_21c\_23 | Hydrolase | Enzyme | Yes |
| 53 | 16- 2\_esterase\_21c\_16 | Hydrolase | Enzyme | Yes |
| 54 | 17- 2\_esterase\_13c\_22 | Hydrolase | Enzyme | Yes |
| 55 | 18- 2\_esterase\_8c\_8 | Hydrolase | Enzyme | Yes |
| 56 | 19- 2\_esterase\_6c\_5 | Hydrolase | Enzyme | Yes |
| 57 | 20- 2\_esterase\_3c\_18 | Hydrolase | Enzyme | Yes |
| 58 | 22- 2\_αβhydrolase\_21c\_9 | Hydrolase | Enzyme | Yes |
| 59 | 24-2\_αβhydrolase\_4c\_6 | Hydrolase | Enzyme | Yes |
| 60 | 25-3\_lipase\_1c\_6 | Hydrolase | Enzyme | Yes |
| 61 | 26-3\_lipase\_1c\_23 | Hydrolase | Enzyme | Yes |
| 62 | 27-3\_lipase\_3c\_12 | Hydrolase | Enzyme | Yes |
| 63 | 29-3\_lipase\_51c\_6 | Hydrolase | Enzyme | Yes |
| 64 | 30-3\_αβhydrolase\_1c\_37 | Hydrolase | Enzyme | Yes |
| 65 | 31-3\_αβhydrolase\_2c\_44 | Hydrolase | Enzyme | Yes |
| 66 | 32-3\_αβhydrolase\_3c\_14 | Hydrolase | Enzyme | Yes |
| 67 | 34-3\_αβhydrolase\_16c\_31 | Hydrolase | Enzyme | Yes |
| 68 | 35-3\_αβhydrolase\_19c\_35 | Hydrolase | Enzyme | Yes |
| 69 | 36-3\_αβhydrolase\_20c\_2 | Hydrolase | Enzyme | Yes |
| 70 | 37-3\_αβhydrolase\_20c\_11 | Hydrolase | Enzyme | Yes |
| 71 | 38-3\_αβhydrolase\_22c\_10 | Hydrolase | Enzyme | Yes |
| 72 | 39- 3\_αβhydrolase\_23c\_15 | Hydrolase | Enzyme | Yes |
| 73 | 40- 3\_αβhydrolase\_29c\_14 | Hydrolase | Enzyme | Yes |
| 74 | 41- 3\_esterase\_2c\_12 | Hydrolase | Enzyme | Yes |
| 75 | 42- 3\_esterase\_4c\_10 | Hydrolase | Enzyme | Yes |
| 76 | 43- 3\_esterase\_4c\_27 | Hydrolase | Enzyme | Yes |
| 77 | 45- 3\_esterase\_10c\_9 | Hydrolase | Enzyme | Yes |
| 78 | 46- 3\_esterase\_14c\_25 | Hydrolase | Enzyme | Yes |
| 79 | 47- 3\_esterase\_15c\_26 | Hydrolase | Enzyme | Yes |
| 80 | 49- 3\_esterase\_16c\_36 | Hydrolase | Enzyme | Yes |
| 81 | 50- 3\_esterase\_20c\_28 | Hydrolase | Enzyme | Yes |
| 82 | 51- 3\_esterase\_24c\_11 | Hydrolase | Enzyme | Yes |
| 83 | 52-3\_esterase\_29c\_9 | Hydrolase | Enzyme | Yes |
| 84 | 54-3\_esterase\_51c\_6 | Hydrolase | Enzyme | Yes |
| 85 | 55-3\_esterase\_65c\_2 | Hydrolase | Enzyme | Yes |
| 86 | 60-4\_esterase\_9c\_19 | Hydrolase | Enzyme | Yes |
| 87 | 64- 4\_esterase\_24c\_3 | Hydrolase | Enzyme | Yes |
| 88 | 67- 4\_esterase\_326c\_1 | Hydrolase | Enzyme | Yes |
| 89 | 71-4\_αβhydrolase\_8c\_31 | Hydrolase | Enzyme | Yes |
| 90 | 72- 4\_αβhydrolase\_10c\_19 | Hydrolase | Enzyme | Yes |
| 91 | 75- 4\_αβhydrolase\_18c\_8 | Hydrolase | Enzyme | Yes |
| 92 | 79- 4\_αβhydrolase\_33c\_10 | Hydrolase | Enzyme | Yes |
| 93 | 33 -3\_αβhydrolase\_13c\_2 | Hydrolase | Enzyme | Yes |
| 94 | 36- 3\_αβhydrolase\_20c\_2 | Hydrolase | Enzyme | Yes |
| 95 | 37- 3\_αβhydrolase\_20c\_11 | Hydrolase | Enzyme | Yes |
| 96 | 48- 3\_esterase\_16c\_29 | Hydrolase | Enzyme | Yes |
| 97 | 53- 3\_esterase\_50c\_1 | Hydrolase | Enzyme | Yes |
| 98 | 56- 4\_lipase\_1c\_22 | Hydrolase | Enzyme | Yes |
| 99 | 57- 4\_lipase\_23c\_6 | Hydrolase | Enzyme | Yes |
| 100 | 61- 4\_esterase\_9c\_28 | Hydrolase | Enzyme | Yes |
| 101 | 63- 4\_esterase\_24c\_4 | Hydrolase | Enzyme | Yes |
| 102 | 70-4\_αβhydrolase\_7c\_19 | Hydrolase | Enzyme | Yes |
| 103 | 75-4\_αβhydrolase\_18c\_8 | Hydrolase | Enzyme | Yes |
| 104 | 77- 4\_αβhydrolase\_20c\_20 | Hydrolase | Enzyme | Yes |
| 105 | 28\_3\_lipase\_7c\_26 | Hydrolase | Enzyme | Yes |
| 106 | 56\_4\_lipase\_1c\_22 | Hydrolase | Enzyme | Yes |
| 107 | 58\_4\_lipase\_23c\_23 | Hydrolase | Enzyme | Yes |
| 108 | 63\_4\_esterase\_24c\_4 | Hydrolase | Enzyme | Yes |
| 109 | 66\_4\_esterase\_280c\_1 | Hydrolase | Enzyme | Yes |
| 110 | 68\_4\_esterase\_806c\_1 | Hydrolase | Enzyme | Yes |
| 111 | 69\_4\_αβhydrolase\_6c\_31 | Hydrolase | Enzyme | Yes |
| 112 | 71\_4\_αβhydrolase\_8c\_31 | Hydrolase | Enzyme | Yes |
| 113 | 76\_4\_αβhydrolase\_18c\_13 | Hydrolase | Enzyme | Yes |
| 114 | Est5E5 | Hydrolase | Enzyme | Yes |
| 115 | LipS | Hydrolase | Enzyme | Yes |
| 116 | LipT | Hydrolase | Enzyme | Yes |
| 117 | PET2 | Hydrolase | Enzyme | Yes |
| 118 | PET6 | Hydrolase | Enzyme | Yes |
| 119 | PET12 | Hydrolase | Enzyme | Yes |
| 120 | PET27 | Hydrolase | Enzyme | Yes |
| 121 | PET28 | Hydrolase | Enzyme | Yes |
| 122 | PET29 | Hydrolase | Enzyme | Yes |
| 123 | PET30 | Hydrolase | Enzyme | Yes |
| 124 | PET30ΔPorC | Hydrolase | Enzyme | Yes |
| 125 | PET30\_doubleMutation | Hydrolase | Enzyme | Yes |
| 126 | PET30\_F80Y | Hydrolase | Enzyme | Yes |
| 127 | PET30\_Y178W mutant | Hydrolase | Enzyme | Yes |
| 128 | PET31 | Hydrolase | Enzyme | Yes |
| 129 | PET38 | Hydrolase | Enzyme | Yes |
| 130 | PET53 | Hydrolase | Enzyme | Yes |
| 131 | PET57 | Hydrolase | Enzyme | Yes |
| 132 | PET58 | Hydrolase | Enzyme | Yes |
| 133 | PET59 | Hydrolase | Enzyme | Yes |
| 134 | Lip6 | Ester-hydrolase | Enzyme | No |
| 135 | Lip7 | Ester-hydrolase | Enzyme | No |
| 136 | Lip8 | Ester-hydrolase | Enzyme | No |
| 137 | meth4 | Ester-hydrolase | Enzyme | No |
| 138 | meth5 | Ester-hydrolase | Enzyme | No |
| 139 | meth6 | Ester-hydrolase | Enzyme | No |
| 140 | Polym1 | Ester-hydrolase | Enzyme | No |
| 141 | Polym2 | Ester-hydrolase | Enzyme | No |
| 142 | Polym3 | Ester-hydrolase | Enzyme | No |
| 143 | ami1 | Ester-hydrolase | Enzyme | No |
| 144 | ami2 | Ester-hydrolase | Enzyme | No |
| 145 | ami3 | Ester-hydrolase | Enzyme | No |
| 146 | ami4 | Ester-hydrolase | Enzyme | No |
| 147 | ami5 | Ester-hydrolase | Enzyme | No |
| 148 | ami6 | Ester-hydrolase | Enzyme | No |
| 149 | amy1 | Ester-hydrolase | Enzyme | No |
| 150 | amy2 | Ester-hydrolase | Enzyme | No |
| 151 | amy3 | Ester-hydrolase | Enzyme | No |
| 152 | amy4 | Ester-hydrolase | Enzyme | No |
| 153 | hyal1 | Ester-hydrolase | Enzyme | No |
| 154 | hyal2 | Ester-hydrolase | Enzyme | No |
| 155 | hyal3 | Ester-hydrolase | Enzyme | No |
| 156 | hyal4 | Ester-hydrolase | Enzyme | No |
| 157 | hyal5 | Ester-hydrolase | Enzyme | No |
| 158 | hyal6 | Ester-hydrolase | Enzyme | No |
| 159 | hyal8 | Ester-hydrolase | Enzyme | No |
| 160 | hyal9 | Ester-hydrolase | Enzyme | No |
| 161 | hyal10 | Ester-hydrolase | Enzyme | No |
| 162 | hyal14 | Ester-hydrolase | Enzyme | No |
| 163 | calB | Ester-hydrolase | Enzyme | No |
| 164 | EH108 | Ester-hydrolase | Enzyme | No |
| 165 | EH115 | Ester-hydrolase | Enzyme | No |
| 166 | EH12 | Ester-hydrolase | Enzyme | No |
| 167 | EH124 | Ester-hydrolase | Enzyme | No |
| 168 | EH127 | Ester-hydrolase | Enzyme | No |
| 169 | EH17 | Ester-hydrolase | Enzyme | No |
| 170 | EH33 | Ester-hydrolase | Enzyme | No |
| 171 | EH36 | Ester-hydrolase | Enzyme | No |
| 172 | EH37 294S | Ester-hydrolase | Enzyme | No |
| 173 | EH37 36F | Ester-hydrolase | Enzyme | No |
| 174 | EH37 36H | Ester-hydrolase | Enzyme | No |
| 175 | EH37 36M | Ester-hydrolase | Enzyme | No |
| 176 | EH37 K62I | Ester-hydrolase | Enzyme | No |
| 177 | EH37 N214I | Ester-hydrolase | Enzyme | No |
| 178 | EH37 P88G | Ester-hydrolase | Enzyme | No |
| 179 | EH37I41W | Ester-hydrolase | Enzyme | No |
| 180 | EH38 | Ester-hydrolase | Enzyme | No |
| 181 | EH4 | Ester-hydrolase | Enzyme | No |
| 182 | EH5 | Ester-hydrolase | Enzyme | No |
| 183 | EH60 | Ester-hydrolase | Enzyme | No |
| 184 | EH63 | Ester-hydrolase | Enzyme | No |
| 185 | EH69 | Ester-hydrolase | Enzyme | No |
| 186 | EH92 | Ester-hydrolase | Enzyme | No |
| 187 | ESTA7 | Ester-hydrolase | Enzyme | No |
| 188 | MetaMorf\_TR2E2 | Ester-hydrolase | Enzyme | No |
| 189 | 6220-AD\_first\_selected | Ester-hydrolase | Enzyme | No |
| 190 | 8301-AD\_seq\_5 | Ester-hydrolase | Enzyme | No |
| 191 | 9409-AD\_second\_selected | Ester-hydrolase | Enzyme | No |
| 192 | 6160-AD\_seq\_4 | Ester-hydrolase | Enzyme | No |
| 193 | 2246-AD\_seq\_10 | Ester-hydrolase | Enzyme | No |
| 194 | EH3-CSIC05 | Ester-hydrolase | Enzyme | No |
| 195 | MetaMorf\_TR2E1 | Ester-hydrolase | Enzyme | No |
| 196 | MetaMorf\_TR2E3 | Ester-hydrolase | Enzyme | No |
| 197 | MetaMorf\_TR2E4 | Ester-hydrolase | Enzyme | No |
| 198 | MetaMorf\_TR2E5 | Ester-hydrolase | Enzyme | No |
| 199 | MetaMorf\_TR2E6 | Ester-hydrolase | Enzyme | No |
| 200 | MetaMorf\_TR2E7 | Ester-hydrolase | Enzyme | No |
| 201 | MetaMorf\_TR2E8 | Ester-hydrolase | Enzyme | No |
| 202 | MetaMorf\_TR2E9 | Ester-hydrolase | Enzyme | No |
| 203 | MetaMorf\_TR2E10 | Ester-hydrolase | Enzyme | No |
| 204 | MetaMorf\_TR2E11 | Ester-hydrolase | Enzyme | No |
| 205 | MetaMorf\_TR2E12 | Ester-hydrolase | Enzyme | No |
| 206 | MetaMorf\_TR2E13 | Ester-hydrolase | Enzyme | No |
| 207 | MetaMorf\_TR2E14 | Ester-hydrolase | Enzyme | No |
| 208 | MetaMorf\_TR2E15 | Ester-hydrolase | Enzyme | No |
| 209 | MetaMorf\_TR2E16 | Ester-hydrolase | Enzyme | No |
| 210 | MetaMorf\_TR2E17 | Ester-hydrolase | Enzyme | No |
| 211 | MetaMorf\_TR2E18 | Ester-hydrolase | Enzyme | No |
| 212 | MetaMorf\_TR2E19 | Ester-hydrolase | Enzyme | No |
| 213 | EH3I244L | Ester-hydrolase | Enzyme | No |
| 214 | EH3I244F | Ester-hydrolase | Enzyme | No |
| 215 | EH7F147A | Ester-hydrolase | Enzyme | No |
| 216 | EH7Y334S | Ester-hydrolase | Enzyme | No |
| 217 | EH0 E226A | Ester-hydrolase | Enzyme | No |
| 218 | EH0 E226G | Ester-hydrolase | Enzyme | No |
| 219 | EH0 E226Q | Ester-hydrolase | Enzyme | No |
| 220 | EH0 Y223A | Ester-hydrolase | Enzyme | No |
| 221 | EH0 Y223W | Ester-hydrolase | Enzyme | No |
| 222 | EH0 P46A | Ester-hydrolase | Enzyme | No |
| 223 | EH0 E226A+I255A | Ester-hydrolase | Enzyme | No |
| 224 | EH0 E226A+Y223A | Ester-hydrolase | Enzyme | No |
| 225 | EH0 E226A+Y119A | Ester-hydrolase | Enzyme | No |
| 226 | EH0 E226A+P46A | Ester-hydrolase | Enzyme | No |
| 227 | EH0 E226A+P46A+Y119A | Ester-hydrolase | Enzyme | No |
| 228 | Hya\_Isolate 2 | Hyaluronidase | Isolate | Yes |
| 229 | Hya\_Isolate 3 | Hyaluronidase | Isolate | Yes |
| 230 | Hya\_Isolate 4 | Hyaluronidase | Isolate | Yes |
| 231 | Hya\_Isolate 5 | Hyaluronidase | Isolate | Yes |
| 232 | *(Halo)-Pseudomonas aestusnigri* | Lipase, cutinase, oxidoreductase | Isolate | Yes |
| 234 | *(Halo)-Pseudomonas bauzanensis* | Esterase, cutinase | Isolate | Yes |
| 235 | *(Halo)-Pseudomonas litoralis* | Esterase, cutinase | Isolate | Yes |
| 236 | *(Halo)-Pseudomonas oceani* | Esterase, cutinase, oxidoreductase | Isolate | Yes |
| 237 | N\_RED | Lipase | Isolate | Yes |
| 238 | IST\_2 | Lipase | Isolate | Yes |
| 239 | IST\_83 | Lipase, esterase, amylase | Isolate | Yes |
| 240 | IST\_14 | Lipase | Isolate | Yes |
| 241 | IST\_232B | Lipase | Isolate | Yes |
| 242 | IST\_457 | Lipase | Isolate | Yes |
| 243 | IST\_700A | Amylase, oxidoreductase | Isolate | Yes |
| 245 | IST\_700B | Protease | Isolate | Yes |
| 246 | IST\_756 | Hyaluronidase | Isolate | Yes |
| 247 | IST\_757 | Hyaluronidase | Isolate | Yes |
| 248 | IST\_758 | Hyaluronidase | Isolate | Yes |
| 249 | IST\_759 | Hyaluronidase | Isolate | Yes |
| 250 | V4 | Hyaluronidase | Isolate | Yes |
| 251 | BNX-91 | Hyaluronidase | Isolate | Yes |

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