

*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

Final ID: 101000327

### 23/11/2021

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First round of functional screens completed

MS9

## Document information sheet

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| --- | --- |
| **Work package:** | WP3, Activity-based bio-prospecting for enzymes |
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| **Document version:** | 1 |
| **Date:** | 23.11.2021 |
| **Starting date:** | 01/06/2021 |
| **Duration:** | 36 months |
| **Lead beneficiary:** | Bangor |
| **Participant(s):** | Bangor, CSIC, UHAM, UDUS, IST-ID, CNR |
| **Dissemination Level:** | - |
| **Type** | Report, materials |
| **Due date (months)** | 6 |
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# FIRST ROUND OF FUNCTIONAL SCREENS COMPLETED

## 1. Introduction

Materials available – this milestone will attest the realisation of the first screens of available bio-resources.

## 2. Summary of the screen effort

By applying a number of protocols extensively described in the deliverable D3.2 and in silico methods, a number of bio-resources (enzymes, clones, microbes, microbial communities) were screened and selected. In details:

382 new bio-resources (strains: 191; clones: 3; pure enzymes: 188) were found positive for activities:

* Hydrolases (esterase/lipase/cutinase): 134
* Hydrolases (proteases): 48
* Hyaluronidases: 3
* Oxidoreductases: 58
* Others (proteases, amylases, etc.): 139

Among them, 307 were identified by functional screens by partners involved in WP3, as listed below.

## 3. IST-ID

Among the bacterial isolates listed in D3.1 and using the agar-based protocols listed in D3.2, 40 isolates with lipase/esterase activity (**Table 1**), 48 isolates with protease activity (**Table 2**), 46 isolates with inulinase activity (**Table 3**), 48 isolates with amylase activity (**Table 4**) and 7 isolates with transaminase activity (**Table 5**) were identified. In most cases, the enzymes were active at 30 and 37°C. These enzymes will be used in the detergent-related experiments. Screening for hyaluronidase activity is currently being conducted.

**Table 1.** Isolates with lipase activity at 30 ⁰C.

|  | Isolate # | Lipase, 30 ⁰C |
| --- | --- | --- |
| *Bacillus subtilis spizizenii* | 83 | ++ |
| *Not identified* | 422 | ++ |
| *Bacillus circulans* | 435 | ++ |
| *Bacillus licheniformis* | 603 | ++ |
| *Bacillus pumilus* | 637 | ++ |
| *Bacillus mycoides* | 82 | + |
| *Bacillus longisporus* | 110 | + |
| *Bacillus longisporus* | 145 | + |
| *Bacillus longisporus* | 174 | + |
| *Bacillus longisporus* | 177 | + |
| *Bacillus longisporus* | 217 | + |
| *Bacillus longisporus* | 225 | + |
| *Bacillus longisporus* | 231 | + |
| *Bacillus pumilus* | 323 | + |
| *Bacillus megaterium* | 348 | + |
| *Bacillus megaterium* | 349 | + |
| *Bacillus megaterium* | 374 | + |
| *Bacillus subtilis* | 375 | + |
| *Bacillus subtilis spizizenii* | 381 | + |
| *Bacillus megaterium* | 383 | + |
| *Bacillus megaterium* | 384 | + |
| *Not identified* | 389 | + |
| *Bacillus pumilus* | 390 | + |
| *Bacillus megaterium* | 394 | + |
| *Bacillus megaterium* | 399 | + |
| *Arthrobacter globiformis* | 431 | + |
| *Bacillus licheniformis* | 481 | + |
| *Bacillus megaterium* | 484 | + |
| *Brevibacillus centrospor* | 562 | + |
| *Alcaligenes faecalis* | 563 | + |
| *Bacillus licheniformis* | 564 | + |
| *Bacillus longisporus* | 568 | + |
| *Bacillus pumilus* | 572 | + |
| *Bacillus pumilus* | 575 | + |
| *Bacillus licheniformis* | 594 | + |
| *Bacillus longisporus* | 641 | + |
| *Bacillus gibsonii* | 651 | + |
| *Bacillus longisporus* | 655 | + |
| *Bacillus longisporus* | 671 | + |
| *Bacillus longisporus* | 689 | + |

**Table 2.** Isolates with protease activity at 30 and 37 ⁰C.

|  | Isolate # | Protease, 30 ⁰C | Protease, 37 ⁰C |
| --- | --- | --- | --- |
| *Bacillus longisporus* | 217 | + | ++ |
| *Bacillus longisporus* | 225 | + | ++ |
| *Bacillus cereus* | 347 | + | ++ |
| *Bacillus subtilis spizizenii* | 381 | + | ++ |
| *Bacillus thuringiensis* | 382 | + | ++ |
| *Bacillus megaterium* | 394 | + | ++ |
| *Bacillus megaterium* | 399 | + | ++ |
| *Bacillus megaterium* | 484 | + | ++ |
| *Bacillus pumilus* | 572 | + | ++ |
| *Bacillus licheniformis* | 594 | + | ++ |
| *Bacillus longisporus* | 641 | + | ++ |
| *Bacillus longisporus* | 655 | + | ++ |
| *Alcaligenes faecalis* | 634 |  | ++ |
| *Virgibacillus pantothenticus* | 596 | ++ | + |
| *Bacillus mycoides* | 82 | + | + |
| *Bacillus subtilis spizizenii* | 83 | + | + |
| *Bacillus longisporus* | 110 | + | + |
| *Bacillus longisporus* | 145 | + | + |
| *Bacillus longisporus* | 174 | + | + |
| *Bacillus longisporus* | 177 | + | + |
| *Bacillus longisporus* | 231 | + | + |
| *Bacillus pumilus* | 323 | + | + |
| *Bacillus megaterium* | 348 | + | + |
| *Bacillus megaterium* | 349 | + | + |
| *Bacillus megaterium* | 374 | + | + |
| *Bacillus subtilis* | 375 | + | + |
| *Bacillus megaterium* | 383 | + | + |
| *Bacillus megaterium* | 384 | + | + |
| *Not identified* | 389 | + | + |
| *Not identified* | 422 | + | + |
| *Bacillus licheniformis* | 481 | + | + |
| *Brevibacillus centrospor* | 562 | + | + |
| *Alcaligenes faecalis* | 563 | + | + |
| *Bacillus licheniformis* | 564 | + | + |
| *Bacillus longisporus* | 568 | + | + |
| *Bacillus pumilus* | 575 | + | + |
| *Bacillus licheniformis* | 603 | + | + |
| *Bacillus pumilus* | 637 | + | + |
| *Bacillus gibsonii* | 651 | + | + |
| *Bacillus marisflavi* | 668 | + | + |
| *Bacillus longisporus* | 671 | + | + |
| *Bacillus longisporus* | 689 | + | + |
| *Alcaligenes faecalis* | 266 | ++ |  |
| *Bacillus thuringiensis* | 346B | ++ |  |
| *Bacillus circulans* | 370 | + |  |
| *Bacillus pumilus* | 390 | + |  |
| *Arthrobacter globiformis* | 431 | + |  |
| *Bacillus circulans* | 435 | + |  |

**Table 3.** Isolates with inulinase activity at 30 and 37 ⁰C.

|  | Isolate # | Inulinase, 30 ⁰C | Inulinase, 37 ⁰C |
| --- | --- | --- | --- |
| *Bacillus subtilis spizizenii* | 83 | + | ++ |
| *Bacillus longisporus* | 217 | + | ++ |
| *Bacillus longisporus* | 225 | + | ++ |
| *Bacillus circulans* | 370 | ++ | ++ |
| *Bacillus thuringiensis* | 382 | ++ | ++ |
| *Bacillus mycoides* | 82 | + | + |
| *Bacillus longisporus* | 110 | + | + |
| *Bacillus longisporus* | 145 | + | + |
| *Bacillus longisporus* | 174 | + | + |
| *Bacillus longisporus* | 177 | + | + |
| *Bacillus longisporus* | 231 | + | + |
| *Bacillus pumilus* | 323 | + | + |
| *Bacillus cereus* | 347 | ++ | + |
| *Bacillus megaterium* | 348 | + | + |
| *Bacillus megaterium* | 349 | + | + |
| *Bacillus megaterium* | 374 | + | + |
| *Bacillus subtilis* | 375 | + | + |
| *Bacillus subtilis spizizenii* | 381 | + | + |
| *Bacillus megaterium* | 383 | + | + |
| *Bacillus megaterium* | 384 | + | + |
| *Not identified* | 389 | + | + |
| *Bacillus megaterium* | 394 | + | + |
| *Bacillus megaterium* | 399 | + | + |
| *Not identified* | 422 | + | + |
| *Bacillus circulans* | 435 |  | + |
| *Bacillus licheniformis* | 481 | ++ | + |
| *Bacillus megaterium* | 484 | + | + |
| *Brevibacillus centrospor* | 562 | + | + |
| *Alcaligenes faecalis* | 563 | + | + |
| *Bacillus licheniformis* | 564 | + | + |
| *Bacillus longisporus* | 568 | + | + |
| *Bacillus pumilus* | 572 | + | + |
| *Bacillus pumilus* | 575 | + | + |
| *Bacillus licheniformis* | 594 | + | + |
| *Virgibacillus pantothenticus* | 596 |  | + |
| *Bacillus licheniformis* | 603 | + | + |
| *Bacillus pumilus* | 637 | + | + |
| *Bacillus longisporus* | 641 | + | + |
| *Bacillus gibsonii* | 651 | + | + |
| *Bacillus longisporus* | 655 | + | + |
| *Bacillus marisflavi* | 668 | ++ | + |
| *Bacillus longisporus* | 671 | + | + |
| *Bacillus longisporus* | 689 | ++ | + |
| *Bacillus pumilus* | 390 | ++ |  |
| *Arthrobacter globiformis* | 431 | + |  |
| *Bacillus thuringiensis* | 346B | ++ |  |

**Table 4**. Isolates with amylase activity at 30 and 37 ⁰C.

|  | Isolate # | Amylase, 30 ⁰C | Amylase 37 ⁰C |
| --- | --- | --- | --- |
| *Bacillus mycoides* | 82 | ++ | ++ |
| *Virgibacillus pantothenticus* | 596 | + | ++ |
| *Bacillus subtilis spizizenii* | 83 | + | + |
| *Bacillus longisporus* | 110 | + | + |
| *Bacillus longisporus* | 145 | + | + |
| *Bacillus longisporus* | 174 | + | + |
| *Bacillus longisporus* | 177 | + | + |
| *Bacillus longisporus* | 217 | + | + |
| *Bacillus longisporus* | 225 | + | + |
| *Bacillus longisporus* | 231 | + | + |
| *Bacillus pumilus* | 323 | + | + |
| *Bacillus cereus* | 347 | + | + |
| *Bacillus megaterium* | 348 | + | + |
| *Bacillus megaterium* | 349 | + | + |
| *Bacillus megaterium* | 374 | + | + |
| *Bacillus subtilis* | 375 | + | + |
| *Bacillus subtilis spizizenii* | 381 | + | + |
| *Bacillus megaterium* | 383 | + | + |
| *Bacillus megaterium* | 384 | + | + |
| *Not identified* | 389 | + | + |
| *Bacillus megaterium* | 394 | + | + |
| *Bacillus megaterium* | 399 | + | + |
| *Not identified* | 422 | + | + |
| *Bacillus licheniformis* | 481 | + | + |
| *Bacillus megaterium* | 484 | + | + |
| *Brevibacillus centrospor* | 562 | + | + |
| *Alcaligenes faecalis* | 563 | + | + |
| *Bacillus licheniformis* | 564 | + | + |
| *Bacillus longisporus* | 568 | + | + |
| *Bacillus pumilus* | 572 | + | + |
| *Bacillus pumilus* | 575 | + | + |
| *Bacillus licheniformis* | 594 | + | + |
| *Bacillus licheniformis* | 603 | + | + |
| *Bacillus pumilus* | 637 | + | + |
| *Bacillus longisporus* | 641 | + | + |
| *Bacillus gibsonii* | 651 | + | + |
| *Bacillus longisporus* | 655 | + | + |
| *Bacillus marisflavi* | 668 | + | + |
| *Bacillus longisporus* | 671 | + | + |
| *Bacillus longisporus* | 689 | + | + |
| *Alcaligenes faecalis* | 266 | ++ |  |
| *Bacillus circulans* | 370 | ++ |  |
| *Bacillus thuringiensis* | 382 | ++ |  |
| *Bacillus pumilus* | 390 | + |  |
| *Arthrobacter globiformis* | 431 | + |  |
| *Bacillus circulans* | 435 | ++ |  |
| *Alcaligenes faecalis* | 634 | ++ |  |
| *Bacillus thuringiensis* | 346B | + |  |

**Table 5**. Isolates with transaminase activity at 30 and 37 ⁰C.

|  | Isolate # | Transaminase, 30 °C |
| --- | --- | --- |
| *Bacillus longisporus* | 225 | ++ |
| *Not identified* | 232 | ++ |
| *Marinobacter psychrophilus* | 240 | ++ |
| *Lacticigenium naphtae* | 256 | ++ |
| *Aeromonas caviae* | 257 | ++ |
| *Not identified* | 521 | ++ |
| *Not identified* | 523 | ++ |

## 4. CSIC

A set of 3 positive clones 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.

## 5. Bangor

Using assays available in the D3.2 (namely, protocol 2.4. Agar plate tributyrin screening esterase-lipase assay, page 9; protocol 3.3. Agar plate polyesterases screening assay with PET or PLA polymers, page 13; protocol 3.10 Agar plate oxidoreductases (laccases/oxidases) screening assay, page 15), Bangor has done the 1st round of screening of cloned putative ester hydrolases from *Thermoleophilum album*, Ischia hot vents, 37 GHases subcloned from fosmid libraries and cloned from reference genomes, 19 laccases (from reference genomes). A set of 80 enzymes were found positive by Bangor, from the total enzymes available listed in D3.1, and new ones identified from a number of bio-resources:

* 40 proteins with laccase and peroxidase-catalase activity (cloned and soluble) – see **Table 6**

**Table 6**. Enzymes with laccase and peroxidase-catalase activity identified by BANGOR.

| Protein name (UofT) | Gene name | Uniprot ID | Enzyme family | Microorganism |
| --- | --- | --- | --- | --- |
| 1. AV0060 | Avin\_00060 | C1DFU7 | DyPa | *Azotobacter vinelandii* |
| 2. BG8310 | Bgla\_2g28310 | F2LPM5 | CPb | *Burkholderia gladioli* |
| 3. BG2090 | Bgla\_2g22090 | F2LQE0 | DyP | *Burkholderia gladioli* |
| 4. BG3010 | Bgla\_2g03010 | F2LII3 | DyP | *Burkholderia gladioli* |
| 5. EL4301 | Entcl\_4301 | E3G4R6 | DyP | *Enterobacter ligninolyticus* |
| 6. FC2591 | Francci3\_2591 | Q2J9T9 | DyP | *Frankia casuarinae* |
| 7. FC3404 | Francci3\_3404 | Q2J7I4 | DyP | *Frankia casuarinae* |
| 8. FM1874 | A374\_01874 | I8AMQ8 | CP | *Fictibacillos macauensis* |
| 9. KR2080 | KRH\_22080 | B2GID3 | DyP | *Kocuria rhizophila* |
| 10. KR3680 | KRH\_03680 | B2GG92 | DyP | *Kocuria rhizophila* |
| 11. LP3150 | Lupro\_03150 | A0A0X8G5A1 | CP | *Lutibacter profundi* |
| 12. LP3855 | Lupro\_03855 | A0A109RN44 | CP | *Lutibacter profundi* |
| 13. MS6384 | MSMEG\_6384 | A0R609 | DyP | *Mycolicibacterium smegmatis* |
| 14. NB4530 | O3I\_014530 | K0ETT3 | CP | *Nocardia brasiliensis* |
| 15. NB8595 | O3I\_018595 | K0EPL4 | CP | *Nocardia brasiliensis* |
| 16. PF3257 | PFL\_3257 | Q4KBM1 | CP | *Pseudomonas fluorescens* |
| 17. PF3705 | PFL\_3705 | Q4KAC6 | DyP | *Pseudomonas fluorescens* |
| 18. PF3734 | PFL\_3734 | Q4KA97 | DyP | *Pseudomonas fluorescens* |
| 19. PR9465 | LT40\_09465 | A0A089YV40 | DyP | *Pseudomonas rhizospherae* |
| 20. RJ5773 | RHA1\_RO05773 | Q0W4I5 | DyP | *Rhodococcus jostii* |
| 21. SH8260 | SHJG\_8260 | K2K2M4 | CP | *Streptomyces hygroscopicus* |
| 22. ATC2173 | Atu2215 | A9CI60 | L | *Agrobacterium tumefaciens* |
| 23. ATC2484 | Atu2528 | A9CHP3 | L | *Agrobacterium tumefaciens* |
| 24. BH2082 | BH2082 | Q9KB49 | L | *Bacillus halodurans* |
| 25. BSU0630 | BSU206300 | P07788 | L | *Bacillus subtilis* |
| 26. CV3643 | CV\_3643 | Q7NRY4 | L | *Chromobacterium violaceum* |
| 27. YfiH | *yfiH* | P33644 | L | *Escherichia coli* |
| 28. CueO | *cueO* | P36649 | L | *Escherichia coli* |
| 29. NE0547 | NE0564 | Q82WU7 | L | *Nitrosomas europea* |
| 30. PA2065 | PA2065 | Q9I250 | L | *Pseudomonas aeruginosa* |
| 31. PA4543 | PA4543 | P33663 | L | *Pseudomonas aeruginosa* |
| 32. RHA03388 | RHA1\_ro04302 | Q0S8P3 | L | *Rhodococcus jostii* |
| 33. RHA08016 | RHA1\_ro05419 | Q0S5I7 | L | *Rhodococcus jostii* |
| 34. RP4125 | RPA4145 | Q6N2A5 | L | *Rhodopseudomonas palustris* |
| 35. SF0119 | SF0119 | A0A0H2UWY4 | L | *Shigella flexneri* |
| 36. SM4080 | RA0681 | Q92Z29 | L | *Rhizobium meliloti* |
| 37. SPA0171 | SPA0171 | A0A0H2WLS8 | L | *Salmonella paratyphi* |
| 38. TSTM0165 | STM0168 | Q8ZRS2 | L | *Salmonella typhimurium* |
| 39. TSTM2506 | STM2661 | Q8ZMW9 | L | *Salmonella typhimurium* |
| 40. TTC1624 | TT\_C1624 | Q72H71 | L | *Thermus thermoplilus* |

aDyP, DyP type peroxidases (dye decolorizing peroxidase)

bCP, catalase-peroxidases

c L,Multicopper oxidases and laccases

* 37 proteins with glycoside hydrolase activity (cloned and soluble) – See **Table 7**.

**Table 7**. Enzymes with glycoside hydrolase activity identified by BANGOR.

| Clone | CAZy | BLAST | Plasmid |
| --- | --- | --- | --- |
| 1. F-GEN-102-l2\_ORF1 | GH13 | alpha-amylase | GEN0081 |
| 2. F-GEN-102-O8\_2 | GH3 | GH3 | GEN0084\* |
| 3. F-GEN-102-O8\_3 | GH51 | Alpha-L-arabinofuranosidase domain protein | GEN0085 |
| 4. F-GEN-11-N5\*\_orf1 | GH5 | Endoglucanase GH5 | GEN0095 |
| 5. F-GEN-14-D2\_orf1 | GH13, CBM48 | Alpha-amylase, GH13 | GEN0096 |
| 6. F-GEN-14-D2\_orf2 | GH12 | GH12 | GEN0097 |
| 7. F-GEN-14-D2\_orf5 | GH3 | GH3 | GEN00100 |
| 8. F-GEN-17-G4\_orf3 | GH4 | Beta-glucosidase | GEN00154 |
| 9. F-GEN-17-G4\_orf4 | GH4 | GH4; 6-phospho-beta-glucosidase | GEN00155 |
| 10. F-GEN-18-I12orf102 | GH13 | alpha-amylase (Nterm) | GEN0018 |
| 11. F-GEN-242N20-32 | *GH5* | Xylanase; cellulase GH5 | GEN0033 |
| 12. F-GEN-242N20-35 | GH30 | O-glycosyl hydrolase GH30 | GEN0035 |
| 13. F-GEN-242N20-40 | *GH3* | Peptidase/beta-lactamase | GEN0036 |
| 14. F-GEN-242N20-41 | GH29 | Fucosidase, COG3669 | GEN0037 |
| 15. F-GEN-242N20-42 | GH43 | Beta-xylosidase; GH43; GH43/62/32/68; COG3507 | GEN0038 |
| 16. F-GEN-242N20-46 | *GH109* | GH109 | GEN0040 |
| 17. F-GEN-242N20-47 | *GH35* | Glycosyl hydrolase GH35 | GEN0041 |
| 18. F-GEN-247-J5-orf136 | GH43, CBM32 | Carbohydrate-binding; possible xylosidase | GEN0051 |
| 19. F-GEN-249-L11\_orf1 | GH5 | Cellulase GH5 | GEN0101 |
| 20. F-GEN-249-L11\_orf2 | GH9 | Glycosyl hydrolase | GEN0102 |
| 21. F-GEN-3-D24-orf14 | GH15 | Glucoamylase | GEN0067 |
| 22. F-GEN-78-P21\_orf1 | GH5 | Glycosyl hydrolase GH5 | GEN0116 |
| 23. F-GEN-79-C10\_orf2 | GH5 | Endoglucanase GH5 | GEN0141 |
| 24. F-GEN-82-K9-59 | GH95 | Possible xylanase or sialate O-acetylesterase | GEN0075 |
| 25. F-HLRH-4-C2 | CBM13 | Glycosyl hydrolase | MGS0003 |
| 26. F-M-10-C9\_orf2 | GH12 | Zinc protease, peptidase M16 | MGS0024 |
| 27. F-M-12-B12\_CMC | N/A | O-sialoglycoprotein endopeptidase | MGS26 |
| 28. F-M-15-C4\_2\_CMC | GH84 | Peptidase | MGS30 |
| 29. F-M-8-D1\_2 | GH84 | Aminopeptidase | MGS68 |
| 30. F-Rim-24-A3\_orf67 | GH73 | Amidase | MGS0158; RIM0001 |
| 31. F-Vul-11-M18\_orf1 | CBM5 | Peptidase; hypothetical | MGS0220 |
| 32. L-CMC-Mu-1 | GT51 | Transglicosylase domain | TH13/GlycoT-Mu-1 |
| 33. P-CMC/HAD-Mu-4 | GH65 | Glycerol-3-phosphatase; HAD | MGS0022 |
| 34. P-CMC-UR-1-B11 orf2 | GH73 | Glycosaminidase; peptidoglycan hydrolase | TH25 |
| 35. P-CMC-UR-2-E8-orf1 | GH57 | Glycosyl hydrolase | TH28 |
| 36. P-CMC-UR-2-E8-orf2 | GH77 | GH77; 4-alpha-glucanotransferase | TH29 |
| 37. P-CMC-Ur-8-F7\_orf2 | GH37 | trehalase | MGS0210 |

* 3 thermophilic metagenomic carboxylesterases from Ischia hot vents BBP-enrichment fosmid library (Ischia,Italy) (10,000 clones max) were identified as shown polyesterase activity:
  + IS10: putative α/β hydrolase (314 aa, Chloroflexi), maximal activity at 70 °C
  + IS12: putative α/β hydrolase (318 aa, Chloroflexi), maximal activity at 70 - 80 °C
  + IS11: β-lactamase-like and lipocalin domains (455 aa, Dehalococcoidea), at 80 - 90 °C

## 6. UDUS and UHAM

A set of 88 esterases and lipases, including 16 cutinase-like enzymes, from previous projects have been collected by UDUS and UHAM, and found to be relevant to the project. The positive enzymes were identified from the total enzymes available listed in D3.1, and new ones identified from a number of bio-resources. For details see **Table 8**.

**Table 8**. Enzymes with polyhydrolase activity identified by UDUS and UHAM (the table represents a 96 deep well plate).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | LCC (WCCG) | Psab PE-H | Abo\_LipA (CE02) | Paes TB045 (CE15) | CalB | Est24c11 | TBEc350 | Hyd8c31 | Sma LipA | Aku GDSL1 (CE17) | Hic | Hyd20c2 |
| B | LCC | Paby PE-H | Hyd18c13 | Abo\_LipC (CE09) |  | TBEcIH8 | TBEc321 | Hyd18c8 | Hyd4c6 | Est8c8 | ED30\_ K329E | Lip10c11 |
| C | PETase | Ppac PE-H | TBEc157 | Hyd13c2 | Aku Est3 (CE20) | FsCut | Est9c19 | Lip3c12 | Hyd3c14 | Abo\_LipE (CE10) | PT35\_ E121Q | Est20c28 |
| D | Plit PE-H | Poce PE-H | TBEc310 | Abo\_LipD (CE07) | Est9c28 | Hyd10c19 | TBEc314 | Aku Est2 (CE19) | Abo\_LipG (CE11) | Abo\_LipI (CE12) | Cyc | Est13c9 |
| E | Pbau PE-H | Paes PE-H Y250S | TBEc304 | Hyd33c4 | Lip1c6 | Est29c9 | Hyd20c11 | Paes TB035 (CE13) | 1,4-D#003 | Hyd22c10 | Dim008 | Est30c5 |
| F | Ppel PE-H | Paes PE-H (CE16) | CycTB025 | Abo\_Est3 (CE03) | MHETase | Est51c6 | Aku Est1 (CE18) | TBEc305 | Hyd23c15 | TBEc308 |  | EstP |
| G | POIL-1 PE-H | Pxin PE-H | Hyd7c19 | EV | Dim-008 (CE01) | Paes TB037 (CE14) | Est16c36 | ED30 | Est24c4 | Hyd19c35 | Est65c2 |  |
| H | Abo\_Est7 (CE05) | Paes TB072(CE24) | Dim004 | Abo\_Est1 | Paes TB040 (CE22) | Sep029-D9 | PaesTB074 (CE25) | Dim001 | Paes TB081 (CE23) | Abo Est2 (CE04) | Paes TB001 (CE21) | Abo Est12 (CE08) |

A collection of >60 lipolytic enzymes from agar-plate screenings using tributyrin for detergent-related characterization studies has been compiled. Enrichments were started for hyaluronate degradative activities using materials from slaughterhouse drains. A metagenomic library with the respective DNA in *Escherichia coli* is available and currently screened for hyaluronate degradation.

## 7. CNR

Two hyaluronic-acid degrading isolates have been identified by CNR, when using Evonik’s hyaluronic acid as substrate for screening:

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