



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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21/11/2023

FIRST ROUND OF LAUNDRY TESTS COMPLETED

MS22

CHRISTIAN DEGERING

HENKEL KGaA

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Document information sheet

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First round of laundry tests completed

1. Means of verification

Report available - this milestone will attest the completion and outcomes of the first laundry tests.

2. Report available

On 05 September 2023, the Industry meeting Detergents #4 online meeting (MS Teams) was organized to discuss about the first round of laundry tests with pre-selected enzymes. Below, the minutes of this meeting are provided that demonstrated that the milestone was achieved.

Participants:

✓	Markus Müller (minutes)	CLIB
✓	Patricia Molina	CSIC
✓	Manuel Ferrer	CSIC
✓	Paula Vidal	CSIC
✓	Jan Modregger	Biosynth (formerly Eucodis)
✓	Christian Degering	Henkel
✓	Fabian Falkenberg	Henkel
✓	Rita Corroero	INOFEA
✓	Carla de Carvalho	IST-ID
✓	Pedro Fernandes	IST-ID
✓	Luis Sousa	IST-ID
✓	Stephan Thies	UDUS

General comments

- First three enzyme candidates have been produced at g-scale by Biosynth and shipped to partners
- Keep list of transferred material up-to-date ([OneDrive folder](#))
- Board of enzyme candidates is accessible now in OneDrive (\Exchange Project Consortium\WP6-WP7 Industry meetings\230908 Enzyme candidate status DTC.pptx)
- Status M22 (Henkel) - First round of laundry tests completed in M22 (07/23)
 - Manolo will prepare draft and circulate
- Next Milestone(s): MS27 – First report on product characteristics (CSIC; 03/24)
 - CSIC in charge of first draft, delivery to partners
- Next Deliverable(s): D7.1 – Report on small/medium validation trials of 18 best pre-selected enzymes (CSIC, 03/24)
 - CSIC in charge of first draft, delivery to partners
- pH and temperature essential for small-scale tests and might differ during the reaction time. Therefore, for future experiments, measure pH before and after the reaction and try to keep it constant by buffering or titration.
- Christian: Short-chain fatty acid generates unliked odor (remaining on textile), therefore esterases producing short-chain fatty acids (such as hexanoic acid) are not preferred for the use in detergents.
- Next Industry Meeting will be on 16 January 2024, 10-11:30 h CET

Decisions and To Do's

- CLIB finalises Declaration of Results and sends it to partners for feedback
- 3 new enzymes have been produced by Biosynth and will be shipped to partners during next week:
 - #07 EstLip-PtEst1 (pPichia57ost1_EstLip-PtEst1 #4)
 - #05 EstLip_Dim_#008 (pPichia57ost1_EstLip-Dim-#008 #3)
 - #04 FE_polur1 (pPichia57aMFd_FE-Polur1 #6)

- Biosynth will ship also additional material of the first three enzymes to Henkel (for mid-/full-scale wash trials)
- Next candidates to be produced at 1-10 L scale:
 - #11 PEH_Pform_PE-H (textile application)
 - #01 Kest3
 - #08 EstLip_TBec304
- Carla & Christian: Schedule meeting to discuss unexpected results in assays with Henkel's detergent
- CSIC: Material transfer of optimised enzyme #02 FE_Lip9 to IST-ID & Henkel
 - Sequence transfer to Biosynth for heterologous expression in Pichia
 - Comparative analysis of optimised (FE_Lip9 Val161Ser) vs. original (FE_Lip9) enzyme

Short reports on Objectives

Objective 1: Analytics (IST-ID)

- How did the first g-scale produced candidates from Biosynth perform? -> Verification of initial enzyme performance?
- Does the analysis for detergent enzymes need to be improved? If yes, how?
- Assay establishment for lipase activity of protein extracts of whole cells on soiled textile using own isolates; measurement of absorbance (500 nm)
 - *Psychrobacter celer*: nearly no activity (only slightly on lipstick)
 - *Pseudomonas protegens*: low activity
 - *Serratia quinivorans*: strong activity
- Testing of enzyme samples provided by Biosynth on beef fat soiled cloth and soiled textile:
 - #02 FE_Lip9: low activity
 - #06 EstLip_Paes_TB035: no activity
 - #09 PEH_Paes_PE-H_Y250S: strong activity
- #9: Assays in 5 mL scale with and without Henkel's detergent (beef fat soiled cloth)
 - No activity when used with detergent
 - Visible activity when used in buffer
 - No activity when commercial enzymes are added to detergent
 - Carla & Christian will re-evaluate the cause for these unexpected results
- Comment Stephan: *E. coli* expressed #09 PEH_Paes_PE-H_Y250S enzyme shows higher stabilities than Pichia-expressed enzyme; influence of glycosylation
- IST-ID is currently developing a cloth rubbing assay in small-scale to mimic a washing machine

Objective 1: Analytics (CSIC)

- Tests at CSIC were focussed on FE_Lip9
- Biosynth preparation (in Pichia-expressed) showed 10-fold less activity than own expression (in *E. coli*)
- By computational optimisation, a mutant (FE_Lip9 Val161Ser) was generated with increased stability at 40°C
- CSIC has performed a 10 L scale fermentation and will send material of FE_Lip9 from *E. coli* to Christian & Carla (lyophilised) for comparative activity assays; in addition, the mutant (FE_Lip9 Val161Ser) will be also produced and sent.
- After comments by partners CSIC agreed to prepare a short document summarizing the biochemical features of the enzymes produced by Biosynth, so that all are aware about the conditions under which each enzymes work and are stable, to further proceed for the small-scale tests.

Objective 2: Enzyme production/verification (Biosynth)

- Which enzymes were produced/shipped already by Biosynth?
 - #02, #06, #09
- Which ones are the next candidates?

- #04, #05, #07
- Were new enzyme candidates added to the short list?
 - Not yet, but optimised #02 could be produced (see CSIC-part)
- #03 FE_ID9: No extracellular expression observed in *P. pastoris*
 - 4 different signal peptides were tested, no positive results
 - Testing of alternative expression hosts possible (*Corynebacterium*, *Streptomyces*, ...)
 - Decision: This candidate will for now not be taken further / into other expression hosts as long as we have enough other candidates available. Before a final decision is taken, CSIC has performed a 10 L scale fermentation and will send material of FE_ID9 from *E. coli* to Christian & Carla (lyophilised) for comparative activity assays.
- First screening of next candidates indicate following activities:
 - #11 PEH_Pform_PE-H (textile application) – confirmed (strong)
 - #01 Kest3 – confirmed
 - #08 EstLip_TBec304 – confirmed (strong)
 - #10 PEH_Pbau_PE H – not confirmed

Objective 3: Optimisation of formulation (Biosynth?)

- At what stage of enzyme/application tests different formulations (which ones?) should be tested?
- Was not discussed during the meeting

Objective 4: Application tests (Henkel)

- What kind of wash trials were performed? What were the results?
- Formulation of liquid raw material was adapted to reference lipase: 20% PG in water
- Tested activity (LipaseU/mL); no direct correlation to activity in wash liquor:
 - #02 FE_Lip9: 90
 - #06 EstLip_Paes_TB035: 9
 - #09 PEH_Paes_PE-H_Y250S: 1.700
 - Benchmark Lipase: 71.500
- Tested stability on wash liquor with & without protease
 - #02 FE_Lip9: very low stability
 - Comment Manolo/Paula: observed low stability at 40°C, much better at 30°C, optimised enzyme (see above) might show better results
 - #06 EstLip_Paes_TB035: highly stable
 - #09 PEH_Paes_PE-H_Y250S: low to medium-low stability (< 20 min)
 - Benchmark Lipase: 71.500
 - All tested enzymes are not sensitive to protease activity
- MWT -mini wash trial – 1 mL, stain CS46B, 40°C, 1 h, 600 rpm
 - Strong standard deviations
 - Only #09 PEH_Paes_PE-H_Y250S shows wash performance
- Experience @Henkel in lipase screenings/small-scale assays is limited, therefore establishment of optimal assays with project partners is essential
 - Required amount of enzyme for full-scale wash trial: 50 mg
- Biosynth can send more material of first three candidates to Henkel for conducting first mid-/full-scale (100-200 mL or washing machine) wash trials.

3. Conclusions

First three enzyme candidates have been produced at g-scale by Biosynth and tested by partners for detergent tests. Based on this, we consider Milestone MS22 achieved.