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FuturEnzyme:
Technologies of the Future for Low-Cost Enzymes for Environment-Friendly Products
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FIRST ROUND OF TEXTILE TESTS COMPLETED

MS23

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Document information sheet

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Summary

First round of textile tests completed

1. Means of verification

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

2. Report available

On 11 September 2023, the Industry meeting Textiles #4 online meeting (MS Teams) was organized to discuss about the first round of textile tests, particularly removal of spinning oils and dyes, with pre-selected enzymes. Below, the minutes of this meeting are provided that demonstrated that the milestone was achieved.

Participants:

✓	Peter Golyshin	Bangor
✓	Alexander Iakounine	Bangor
✓	Anna Khusnutdinova	Bangor
✓	Fabrizio Beltrametti	BioC-CheM Solutions
✓	Markus Müller (minutes)	CLIB
✓	Tobias Klement	CLIB
✓	Patricia Molina	CSIC
✓	Manuel Ferrer	CSIC
✓	Paula Vidal	CSIC
✓	Jan Modregger	Biosynth (formerly Eucodis)
✓	Carolina Giunta	INOFEA
✓	Carla de Carvalho	IST-ID
✓	Nazanin Ansari	Schoeller
✓	Roland Lottenbach	Schoeller
✓	Rainer Rösch	Schoeller
✓	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)
- 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
- Four enzyme candidates are free to be tested for textile as well (originally planned only for detergents):
    - #5 EstLip_Dim_#008 (lipase)
    - #6 EstLip_Paes_TB035 (lipase)
    - #7 EstLip_PtEst1 (lipase)
    - #8 EstLip_TBEc304 (lipase)
    - Activity might be lower on textiles (first indications), needs to be tested under realistic application condition; most promising of these candidates for textiles is #5
- Bio_CH has a potent laccase originating from another project
    - Partners (IST-ID, CSIC, Schoeller, INOFEA) should contact Fabrizio to receive samples
    - Information of enzyme will be added to short list (#28)

- Bangor has a new candidate to be added to the list:
    - #29 pVac11 (peroxidase)
    - Sequence has already been provided to Biosynth for production
    - Details and assay protocol available in OneDrive folder

Decisions and To Do's

- CLIB finalises Declaration of Results and sends it to partners for feedback
- Bio_CH / Fabrizio: Provide detailed information on laccase candidate (#28)
    - IST-ID, CSIC, Schoeller, INOFEA: Contact Fabrizio for receiving this candidate
- 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 can ship also additional material of the first three enzymes to Schoeller; Nazanin will contact Jan directly which enzymes are required in which quantities
- Next candidates to be produced at 1-10 L scale:
    - #11 PEH_Pform_PE-H
    - #01 Kest3
    - #08 EstLip_TBEc304
    - #29 pVec11
- Schoeller: Provide IST-ID with new dyeing liquid (as old one gets degraded/chemically changed over time)
- Manolo: Send meeting link to the participant list for 28 September to discuss results obtained by first Schoeller trials and set up of new tests based on results with new material provided by Biosynth (for example #04 FE_polur1)
- CSIC: Material transfer of optimised enzyme #2.2 Lip9 Val161Ser and #3 FE_ID9 to IST-ID, Henkel, Schoeller
    - Transfer of #2.2 Lip9 Val161Ser sequence to Biosynth for heterologous expression in Pichia
    - Comparative analysis of optimised (Lip9 Val161Ser) vs. original (Lip9) enzyme
- Milestones
    - MS23 (Schoeller; 07/23): Manolo will prepare draft and circulate

Short reports on Objectives

Objective 1: Analytics and verification (IST-ID)

- How did the first g-scale produced candidates from Biosynth perform? -> Verification of initial enzyme performance?
- Does the analysis for textile enzymes need to be improved? If yes, how?
- Application 1: Spinning oil removal
    - Stirring challenge in small scale
    - 3D printed prototype is under construction, will be finalised in next weeks
- Application 2: Excess dyestuff removal
    - 2-step process to remove excess dye: 1st concentration in silica particles; 2nd enzymatic degradation of dye color
    - Whole cells (bacteria) and purified enzymes were tested
    - Analysis by UV-VIS and GC-MS
    - No color degradation by lipases #2, #6, #9 and by bacteria (14 and 790) as expected
    - Color degeneration over time -> New dyeing liquid needed from Schoeller
    - Comment Carolina: Laccases decolorises the dye successfully
    - When testing dyed textile (from Schoeller), mass transfer might be limited. Should however be considered in future experiments

    - Benchmark possible by H_2O_2 treatment (to see maximal achievable discoloration) -> will be tested in next experiments
- Serial treatment with different enzymes could be considered, but keep in mind different reaction optima (pH, temperature) might limit simultaneous utilisation in a cocktail
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Objective 1: Analytics and verification (CSIC)

- Application 1:
    - Assay established on lipase activity using NEFA-Kit of Wako (colorimetic detection of fatty acids)
    - Lip9 showed no activity on different textiles, enzyme is instable
    - Mutant was generated: Lip9 Val161Ser; shows increased stability at 40ºC
    - Kit might not measure all fatty acids (mainly measuring long fatty acids), therefore no shorter fatty acids are measured, might underestimate enzyme activities; detailed analysis favourable
    - INOFEA has protocol for contact angle measurement that might be easy and more reliable/flexible -> exchange protocols between INOFEA and CSIC
    - Comment Stephan: Addition of detergents to the reaction mix might help to release longer fatty acids from the textile to be accessible for Kit analysis
    - Benchmark / Commercial-enzyme or treatment not yet established but might help to determine maximal signal
    - IST-ID has performed a full removal of spinning oil from textile -> transfer data/method to CSIC
    - 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.
- Comment Jan: *Corynebacterium*, *Bacillus*, etc. can be used as alternative production host for mutant

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 and Nazanin (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
- Currently no trustable stable assay for in-house verification of expression/activity at hand;
  → crucial for online monitoring during mid-to-large scale production
- Peroxidise and enzymes needing other expression systems will be prioritised
- Single enzyme candidates could also be produced in *E.coli*; CSIC will ship their ID9 enzyme and Lip9 Val161Ser mutant to Henkel, Schoeller, IST-ID, INOFEA for comparative assays

Objective 3: Enzyme immobilisation (INOFEA)

- What immobilisates have been produced/tested?
- How did the immobilisates perform compared to the soluble enzyme?
- Decolorisation tests with silica particles any news?
- Bangor offered assistance in evaluating performance of immobilised enzymes
- Application 2:
    - Testing of commercial laccase shows good results in decolorising silica-concentrated dye
    - Soluble enzyme is faster than immobilised enzyme
    - Specific information needed what optimisation (T-, pH stability) are hard to achieve by protein engineering; those limitations could be overcome by shielding the immobilised enzymes
- Application 1:
    - Partial shielding planned to retain enzyme activity
    - TB035 highly active in pNPB assay, Y250 medium, Lip9 no activity
    - PE-H needs hydrophobic moieties -> engineering of particles
    - In general: good performance in shielding: activity is retained by immobilisation
- Enzyme specification sheet needed with basic informations on enzyme (optimal conditions, stress conditions for industrial applications as engineering targets)
- Use of surfactant is good to remove excess oils; protocol will be shared with IST-ID

Objective 4: Application tests (Schoeller)

- Have recommendations been made by IST-ID/CSIC how to set-up first application tests?
- What type of application tests has been conducted/is planned for the next months?
- Application 1: Spinning oil removal
    - Enzyme recipies received by IST-ID, CSIC
    - Needed 3 g of enzyme per experiment in lab-scale Foulard machine (100-200 mL scale)
    - Starting point for experiments: high enzyme concentration (15 g/L), long incubation time (24 h); if positive, decrease incubation time and enzyme concentration
    - 24 h or 6 h impacting time would be ideal for industry procedures (working shifts)
    - Benchmark is first treated textile sample (that has also been sent to partners)
    - Scheduling of separate meeting on 28. 9. To show and discuss analytics of first run of experiments
    - Pick up [%] = amount of liquid taken up by textile (with regard to initial dry weight)
- Application 2: Excess dyestuff removal
    - No need to fully degrade all ingredients of the dye. If the waste water is cleared visually that is good enough for Schoeller.

3. Conclusions

First five enzyme candidates have been produced at g-scale by Biosynth and tested by partners for textile tests. Based on this, we consider Milestone MS23 achieved.