



The HotZyme Newsletter



SYSTEMATIC SCREENING FOR NOVEL HYDROLASES FROM HOT ENVIRONMENTS

Preface

Dear readers,

Welcome to the second newsletter of the HotZyme-project on Systematic screening for novel hydrolases from hot environments. A selection of research and policy papers can be found on our website <http://hotzyme.com/>

In this newsletter, we present a summary of results, focusing on the research findings of Deliverable 1.3 (Work Package 1), entitled: "New thermophilic isolates with hydrolytic activity" and Deliverable 4.1 (Work Package 4), entitled: " Database set up." This newsletter contains abstracts of these reports and their main conclusions, the full version of which will be available after EC approval (*Note: some deliverables are not for public dissemination, but are for a more restricted audience*).

One of the aims of our newsletters is to provide you with a comprehensive overview of the main body of research carried out, hence in this issue the emphasis is on the Project Deliverables - from work already completed to those still in working progress, to a full list of deliverables – all for the purpose of shedding light on what this project is trying to achieve. Also highlights from recent progress meetings of consortium members are presented.

On behalf of the HotZyme consortium

Haimo Gilbert (EuCRF)
Editor, the HotZyme Newsletter



Website:

For more information about the project, its participating partners and events, please visit the project official website at: <http://hotzyme.com/>

Contribution:

What's your team up to? Please share the breakthroughs and other news with us by writing to – haimo@eu-crf.net

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Deliverable Expectations

The Participating Partners of the Hotzyme project carry the responsibility to submit regular reports on the project's progress and implementation in the forms of deliverables, milestones, periodic reports and final reports.

Since the project's launch in April 2011, a total of 6 scheduled Hotzyme deliverables were submitted to the EU Commission, now pending review. Here is a closer look at the most recently submitted deliverables:

D1.3) New thermophilic isolates with hydrolytic activity (completed by Work Package 1)

23 strains of thermophilic microorganisms able to hydrolyze polymeric substrates were isolated from various volcanic environments (Kamchatka, Kuril Islands, Mongolia hot springs). Filter paper, microcrystalline cellulose, carboxymethyl cellulose, bamboo or corn leaves served as cellulosic substrates. Other polymeric substrates tested were birch xylane, starch, chitin, lignine.

The isolates were in majority anaerobes growing in the absence of oxygen. They belonged to Bacteria or Archaea domains and grew at temperatures from 50 to 90°C. The activity of interest was tested either by visible solution of insoluble substrates in the course of microbial growth, or by special method (zymography) allowing to estimate not only the degradation of polymeric substrate, but also the localization and molecular weight of the hydrolytic enzyme of interest.

(Contributed by Dr. Elizaveta Bonch-Osmolovskaya from INMI)

D4.1) Database set up (Completed by Work Package 4)

This deliverable describes the development of a bioinformatics workflow that performs the storage and annotation of metagenomics samples. The database resulting from the application of the workflow will contain comprehensive information of thermal ecosystem biodiversity and will enable the further investigation of the desired functional properties.

The analysis of metagenomic data begins with the assembly of the small DNA fragments, which are generated from the sequencer (raw reads), to contigs larger sequences. Then, the contigs are used as the main input to gene and protein function prediction algorithms. Although various tools have been developed for these purposes, it yet remains challenging from the computational aspect to handle efficiently the versatile different annotation tasks required. Therefore, the annotation workflow was additionally integrated into the Galaxy platform.

Galaxy is an open-source framework for the integration of computational tools and databases into a cohesive workspace and can be used for data intensive biomedical research. Galaxy provides a user-friendly web interface where users can develop, execute and share workflows of complex analyses that can be repeated on many different datasets, or refactored for different computing purposes. The users have the option of using a public server for their analysis or to install a fully customizable local instance on their own server. The integration of the workflow into Galaxy enables the automatic and easy handling of large amounts of data by every user, regardless of expertise in executing bioinformatic tasks. The resulting database will permit the fast investigation of sequences of interest and the subsequent derivation of putative thermostable enzymatic functions.

(Abstract taken from the original Deliverable 4.1 report 'Database set up', written by Work Package 4 participants)

Deliverables aims and completions:

To ensure that a large-scale integrating project such as the Hotzyme stays on track with its progress over the assigned 48-month contract period, a detailed list of Deliverables and Milestones for each Work Package were set with deadlines for report submissions. For your information, below is the full list of deliverables for the project, which will provide insight into what the project aims to achieve.

Deliverable Number	Deliverable Title
D1.1	Hydrothermal and hot deep subsurface samples from different locations.
D1.2	Nucleic acid preparations from selected environmental samples and enrichment cultures.
D1.3	New thermophilic isolates with hydrolytic activity
D2.1	Metagenomic libraries of thermophilic samples
D2.2	Metagenomic libraries and high-throughput sequencing of new environmental samples collected in WP-1
D3.1	Pipeline set up for analysis of raw data
D3.2	Development of specialized classifier for secreted proteins
D3.3	Development of protein classifier
D4.1	Database set up
D4.2	Taxonomic library database of biodiversity in high temperature ecosystems
D4.3	Gene database of novel thermostable hydrolases
D5.1	Infrastructure for sample tracking and coordination in place for multinational HTS effort
D5.2	Synthesis of substrates suitable for HTS of thermophilic hydrolases
D5.3	Creation of expression libraries in suitable hosts from metagenomics libraries for screening
D5.4	HTS with aim of generating 100 promising lead clones as "hits"
D5.5	Validation of "hits" and prioritization for analysis in WP6
D6.1	Molecular modeling of new hydrolase enzymes
D6.2	Full Enzymatic characterization
D6.3	Crystallization trials with selected hydrolase enzymes
D6.4	Evaluation of stability of selected hydrolase enzymes
D6.5	Crystallographic analysis of selected proteins
D6.6	Production of protein variants by rational design based on modeling and structural analysis
D7.1	Consortium agreement
D7.2 – 7.4	Periodic Report 1-3
D7.5	Final Report
D7.6 – 7.13	6 Month report 1-8
D7.14	Setting a project interactive website
D8.1	Commercialisation/Exploitation plan
D8.2	Training session 1
D8.3	Draft for Dissemination plan for increasing use of project results
D8.4	Training session 2
D8.5	Symposia and other information meetings with young scientists
D8.6	e-newsletters
D9.1	Interest groups and interests
D9.2	Policy-related consideration
D9.3	Potential markets and use

Deliverables submitted to date (in order of scheduled submission):

Note that the deliverables cannot be made public until the EC has approved them at the end of each year of the project. Also some deliverables are not for public dissemination, but are for a more restricted audience.

D1.1) Hydrothermal and hot deep subsurface samples from different locations: Hydrothermal and hot deep subsurface samples from different locations will be obtained, enriched, safely stored and shared between some of the participants. In total we aim to collect around 120 different samples. (WP 1)

D4.1) Database set up: A server with 36 CPU cores, 256 GB RAM and a fast storage machine with 32 TB capacity will be set up at Copenhagen for metagenomic data assembly, storage and sequence analysis. (WP 4)

D5.1) Infrastructure for sample tracking and coordination in place for multinational HTS effort: Infrastructure for sample tracking and coordination will set in place for multinational HTS effort. (WP5)

D1.2) Nucleic acid preparations from selected environmental samples and enrichment cultures: Nucleic acid will be prepared from selected environmental samples and enrichment cultures. Micrograms of DNA or RNA will be obtained. In case of low biomass, the material will be amplified in vitro. (WP 1)

D2.1) Metagenomic libraries of thermophilic samples: Metagenomic libraries of thermophilic samples from Japan, Italy, New Zealand and Iceland (collected before, ready for library construction) will be constructed and sequenced. (WP 2)

D1.3) New thermophilic isolates with hydrolytic activity: New thermophilic organisms with hydrolytic activity will be isolated. We aim to obtain around 50 single isolates or enrichments with hydrolytic activities. (WP 1)

Coming Soon – Deliverables due in the next quarter:

D2.2 - Metagenomic library construction and high-throughput sequencing of new environmental samples collected in WP-1 will be performed to obtain the sequence data.

D3.1 - Pipeline will be set up for analysis of raw read data (assembly, gene finding). The output is a list of likely amino acid sequences that will be used for function prediction.

D3.2 - Specialized classifier for secreted proteins will be developed to predict enzymes that are secreted outside of cells.

D3.3 - A comprehensive set of programs will be combined to predict protein function.

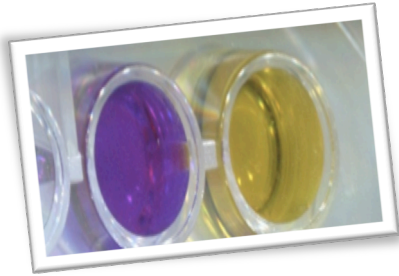
D5.2 - A variety of substrates suitable for HTS of thermophilic hydrolases will be synthesized.

D5.3 - Expression libraries will be constructed from metagenomic libraries for screening in suitable hosts.

D8.1 - Preparing a comprehensive commercialisation and exploitation programme, based on a thorough study of the project ongoing production of results on the one hand and, a mapping endeavour of potential arena toward which the project should target its commercialization effort. The plan will include potential targeted items/ services worthy of commercialisation and potential target markets/ clientele.

Recent WP Progress Meeting Highlights:

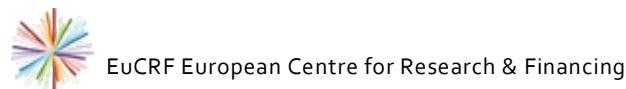
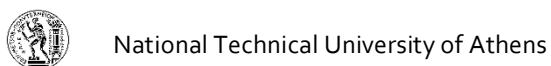
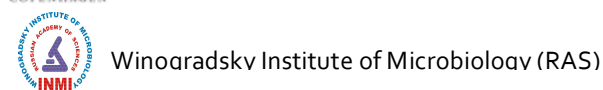
Throughout the first quarter of 2012, HotZyme's consortium of academic and industrial partners has continued to make progress in achieving their respective objectives for the project. Different Work Package leaders requested and hosted teleconference and web meetings giving each work team the opportunity to share their findings:



- **WP2 Teleconference in March** - A progress report teleconference for Work Package 2 (Metagenome library construction and sequencing) was held on the 1st March. The aim of the event has been to provide a comprehensive discussion and update opportunity for the representatives or consultants of Work Package 2 which comprised of participants from University of Copenhagen, Montana State University Bozeman, Stiftelsen Norges Geotekniske Institutt and INMI. Focus of the teleconference has been given to discussion over sequencing capacity and platform of various samples (environmental and isolates) in relation to their next deliverable - Metagenomic libraries and high-throughput sequencing of new environmental samples collected in WP1.
- **March Consortium web meeting** - Consortium members from 12 participating partner groups took part in a web meeting organised by the project coordinator on March 26th. Although the focus of the meeting was on the possible screening methods and status for HotZyme WP5, several other work progress were presented by:

 - Dr. Zalan Szabo from Microdish BV (MBV) summarized work progress of WP5: different screening methods are being tested; enzymes from known organisms have been cloned in different expression organisms; tracking system for sample storage has been established.
 - Dr. Elizaveta Bonch-Osmolovskaya from Winogradsky Institute of Microbiology of the Russian Academy of Sciences (INMI) summarized the work progress of WP1+2: enrichments on different substrates have been made from half of which DNAs have been extracted. Sequencing of 16S RNA gene of different enrichments is on-going. Isolates have been obtained from Xylan, Xanthan Gum, cellulose, Beta-keratin, starch enrichments etc.
 - Peter Menzel from University of Copenhagen (UCPH) and Dr. Aristotelis Chatziioannou from National Technical University of Athens (NTUA) reported their work on WP3 and 4. Further meeting for WP3 and WP4 is arranged for end of April in Athens organized by NTUA.

Hotzyme Partners:



This is a newsletter distributed by the HotZyme project consortium to those interested in our EU FP7 coordination action project on Systematic Screening for Novel Hydrolases from Hot Environments.

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The research project is a large scale integrating project collaborative project - on the systematic screening for novel hydrolases from hot environments.

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