Intenso Newsletter 3/2015

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Editorial:

Dear reader, we would like to introduce you to the third Intenso newsletter. A lot has happened in our project since the last newsletter was published, and we appreciate your interest. The project is now running for 36 months and the partners are still working hard to find suitable solutions to overcome the bottleneck of the downstreaming process. As you can read on our project website we are in contact with another project called “BioIntense”

We hope you like the topics of this newsletter.

Meeting with BioIntense: In September 2015 we met the other project funded in the same call that Intenso is funded in, BioIntense, again. The first meeting was in Lisbon/Portugal in February 2014 where a project member of Biointense presented their project idea to our consortium. This project started in the same year as Intenso and is running for 36 months. This time, a member of the Intenso project was invited to their project meeting. The meeting occurred in the city of Lund/Sweden (pictured the venue of the meeting, the old Bishop’s residence), and provided ample opportunity for both projects to get to know the aims and goals of the other one. If interested, please find the website of BioIntense here: http://www.biointense.nu/ .

Permeabilisation

Plasma membrane permeabilization by pulsed electric field (PEF) treatment (electroporation/electropermeabilization) is an efficient and versatile technique, which nowadays has found numerous applications in molecular biology, medicine and biotechnology. The loss of membrane integrity, which is associated with the induction of an additional transmembrane potential, can be reversible or irreversible depending on electrical parameters, media conductivity and temperature and phase of the cell growth. PEF treatment that induces irreversible electropermeabilization gained large popularity in the last years as a suitable method for large scale extraction of biologically active compounds from microorganisms and plant cells.

In the frame of the INTENSO project the main goal of Sofia University is to evaluate the applicability of PEF treatment for recovery of intracellular recombinant proteins from different yeast species as an alternative method to mechanical disintegration. In all experiments the electrical treatment was performed with monopolar square pulse generators by using a continuous flow chambers, thus creating a homogenous electric field.

The aim was, on one side, to find conditions enabling highly efficient and selective liberation of proteins/enzymes with preserved biological function, and on the other side to demonstrate the possibility to scale up the process.

One of the main problems when using cell permeabilization for recovery of intracellular bio-products from yeast is the yeast cell wall, which is a relatively thick and rigid structure that limits the passage of macromolecules into and out of the cells. The conditions applied for induction of recombinant yeast strains often lead to a further strengthening of the cell walls and to a decreased permeability.

Therefore, to achieve an efficient release of intracellular proteins, a treatment resulting in an increase of cell wall porosity is required in addition to plasma membrane permeabilization.

Our investigations, performed with several yeast systems, demonstrated that PEF treatment has two effects on the yeast cells – it leads to plasma membrane permeabilization but it also provokes changes in the cell wall structure, thus making the cells more sensitive to lytic enzymes. The treatment of electropermeabilized cells with a low concentration of lytic enzyme leads to efficient recovery of proteins without provoking cell lysis. This enabled the development of a procedure, which can be used for selective and efficient recovery of large (450 – 550 kDa) intracellular recombinant proteins from different yeast species (*S. cerevisiae* and *H. polymorpha*). Currently, different strategies to scale up the process are evaluated: increase of the flow rate during pulse application, increase of the concentration of treated suspensions, testing the efficiency at lower lytic enzyme concentration and testing the efficiency of different lytic enzymes with lower price. As the developed procedure leads to relatively selective product liberation, and it is not associated with cell fragmentation and an increase of suspension viscosity, one can expect that the following

downstream processing will be facilitated considerably. Experiments regarding the purification of recombinant proteins obtained by the developed procedure are currently in progress, in collaboration with other partners of INTENSO Project.

Partner changes

The consortium has to record changes. Two partners left the consortium (ERA Biotech and Patheon) and Zip Solutions S.L. joint.

ZIP Solutions is a Biotech SME developing breakthrough proprietary technologies for the production and purification of recombinant proteins.

The company acquired and further developed robust technological platforms that have been widely validated for their efficacy at an initial stage and focuses on their commercial exploitation, both through co-development with customers and through the out-licensing of owned intellectual property. For more information here is the website: http://www.zipsolutions.es

Publications:

The consortium was very busy in publishing. Please find below a list with our latest publications.

Title of the article	DOI
Sample displacement chromatography of plasmid DNA isoforms	10.1016/j.chroma.2015.08.035
Chromatographic Characterization and Process Performance of Column-Packed Anion Exchange Fibrous Adsorbents for High Throughput and High Capacity Bio separations	10.3390/pr3010204
Interactions of Chinese Hamster Ovary (CHO) cell cultures with second generation expanded bed adsorbents	10.1016/j.seppur.2015.02.014
Preparation and characterization of grafted cellulosic fibers and their applications in protein purification	10.1016/j.seppur.2015.01.042
Synthesis and performance of megaporous immobilized metal-ion affinity cryogels for recombinant protein capture and purification	10.1016/j.chroma.2012.11.036
The role of ligands on protein retention in adsorption chromatography: A surface energetics approach	10.1002/jssc.201301338

More information about the project can be found on our website: http://intensoproject.eu/