Tercer Encuentro & Primer Workshop de la Red Argentina de Tecnología Enzimática

Septiembre 8 - 9 - 10, 2021 ▸ VIRTUAL

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Welcome letter

The Argentine Network of Enzyme Technology (RedTEz) was created in 2019 by five researchers from different institutions of our academic system who detected the scarce interaction between those of us who currently work in different fields of enzymology.

The presentations of biochemistry and enzymatic characterization of doctoral and postdoctoral fellows and young researchers are usually scattered in various meetings of different scientific societies. This means that the oral presentations and posters on the subject have a reduce audience, which limit the discussions and the enrichment of results. So, the matrix of plural and shared knowledge that would be essential to take advantage of, is often not generated. We consider that this fact affects the development of the students and the discipline and, in turn, makes interaction with the private sector difficult as there are not specific events.

Technology-based companies are important actors in the economic development since they have a key role in transmitting innovation and knowledge to the productive fabric, generating highly qualified jobs and moving the economy. In this area, the development of enzymes is a promising alternative with great impact, and so promoting the exchange of experiences and knowledge is a priority. With this in mind, we organized a series of meetings with the aim of facilitating the meeting and collaboration between research professionals from Argentina and Latin America, who are working on enzymology and enzyme technology for various applications; to communicate and discuss ideas and results, as well as to cooperate in different current and future projects. We also invited the local productive sector to join this initiative.

During 2020 and in the midst of the pandemic, two virtual meetings were held with speakers from Argentina, Chile and Brazil, which were very well received by the scientific community. We had the presentations of doctors Hebe Dionisi from the National Patagonian Center (CENPAT), Puerto Madryn; Alberto Iglesias, from the Litoral Agrobiotechnology Institute (IAL), Santa Fe; Igor Polikarpov, from the Physics Institute, USP-Sao Carlos; Laura María Isabel López, from CITEC-INTI -UNAJ; María Gabriela Guevara, from IIB-UNMdP and Lorena Wilson from the Enzymatic Biocatalysis Group of the Pontificia Universidad Católica de Valparaíso. We want to acknowledge them all and express our gratitude as they helped us launch the idea and sustain it. With a highly positive balance of both meetings, we decided to face the organization of the 2021 meeting, initially intended as face-to-face and which had to be changed to virtual due to the current situation. We are proud to receive one hundred and sixty participants (with thirty undergraduate scholarship students) and offer six plenary lectures, three round tables, thirty-seven posters and seventeen oral communications.

We also thank REDBIO for its support and collaboration so that we can carry out this meeting and the previous ones and to CONICET and the R + D + i Agency for the financing granted, as well as our commercial sponsors for their support.

We welcome you to the THIRD MEETING OF THE ARGENTINE ENZYMATIC TECHNOLOGY NETWORK and the FIRST WORKSHOP RedTEz.

Matías Asención Diez, Molecular Enzymology Laboratory, IAL (UNL-CONICET).

Martín Blasco, Department of Bioprocesses, Technical Directorate of Biotechnology, INTI National Institute of Industrial Technology.

María Victoria Busi, CEFOBI, Center for Photosynthetic and Biochemical Studies (UNR-CONICET).

Eleonora Campos, IABIMO Institute of Agrobiotechnology and Molecular Biology, UEDD INTA-CONICET.

Hernán Costa, Department of Basic Sciences, National University of Luján (UNLu); Institute of Ecology and Sustainable Development (INEDES-CONICET-UNLu).

Comité Organizador

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Consejo Nacional de Investigaciones Científicas y Técnicas

Agencia I+D+i

Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación

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MICROBIOLOGÍA AGROINDUSTRIAL

ENZI NOVA

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INTA

Ministerio de Agricultura, Ganadería y Pesca Argentina

Instituto Nacional de Tecnología Industrial

INTI

UNIVERSIDAD NACIONAL DE LUJÁN

SAIB

Sociedad Argentina de Investigación en Bioquímica y Biología Molecular

Wednesday September 8

09:00 - 09:05 **Conference welcome**
Conference welcome by Dra. María Victoria Busi and Coordinators of the TEz Network.

09:05 - 10:15 **Plenary session**
Chair: Dra. Eleonora Campos

Industrial and specialty enzymes: perspectives and challenges
Dra. Elba Pinto Da Silva Bon
Chemistry Institute of the Federal University of Rio de Janeiro, Brazil

10:15 - 10:30 Coffee break *at home*

10:30 - 12:00 **Oral communications session I**
Chairs: Dra. Eleonora Campos / Dr. Hernán Costa

EP-2
Biochemical characterization of *Cs*Abf62A, a novel α-L-arabinofuranosidase enzyme from *Cellulomonas* sp. B6
Garrido, M.; Landoni, M.; Topalian, J.; Couto, A.; Wirth, S.; Campos, E.

EP-9
Characterization of SdGA, a cold-adapted and salt-tolerant glucoamylase from *Saccharophagus degradans*
Wayllace, N.M.; Hedin, N.; Busi, M.V.; Gomez-Casati, D.F.

EP-14
CBMs in trans: generating alternatives to improve the catalytic efficiency of enzymes
Busi, M.V.; Grisolía, M.J.; Hedin, N.; Gomez-Casati, D.F.

EP-15
Mining an intertidal sediment metagenome for fucanases for the production of oligosaccharides from brown algae fucoidans
González, J. A.; Ponce, A.; Stortz, C.; Lozada, M.; Dionisi, H.

OLP-1
Carboxylesterase as a nuclear lipase involved in lipid-droplet homeostasis
Lagrutta, L.C.; Trejo S.A.; Ves Losada, A.

12:00 - 14:00 Lunch *at home*

14:00 - 15:15 **Plenary session**
Chair: Dra. María Victoria Busi

Structural insights into the *de novo* biosynthesis of glycogen
Dra. María Elena Carrizo García
Research Centre in Biological Chemistry of Córdoba (CIQUIBIC), National University of Córdoba, Argentina

Thrusday September 9

17:00 - 19:00 **E-Posters sessions at *Gather Town***

17:00 - 17:30 **Session I** *(cont.)*

ED-1
Metal binding affinity and stability of *Bacillus cereus* phospholipase C variants
Di Nardo, L.; Val, D.; Castelli, M.E.; Rasia, R.

EP-1
Characterization of a new type I pullulanase isolated from *Exiguobacterium alkaliphylum*
Castillo, J. de las M.; Bertoneri, A.F.; Caminata Landriel, S.; Costa, H.

EP-3
Enhanced cellulose degradation by the polymeric coupling of a cellulase to a thermostable decameric scaffold: a step towards sustainable energy
Iglesias Rando, M.R.; Gorojovsky, N.; Goldbaum, F.A.; Craig, P.O.

EP-5
Identification of key residues for the functional differentiation of glycosyl hydrolase family 1
Irazoqui, J.M.; Eberhardt, M.F.; Amadio, A.F.

EP-7
Enhancing the *Ruminoccocus albus* 8 cellodextrin phosphorylase to process oligosaccharides
Storani, A.; Guerrero, S.A.; Iglesias, A.A.

EP-9
Characterization of SdGA, a cold-adapted and salt-tolerant glucoamylase from *Saccharophagus degradans*
Wayllace, N.M.; Hedin, N.; Busi, M.V.; Gomez-Casati, D.F.

EP-11
Evaluation of bioinformatic methods for the prediction of stabilizing mutations and its use in the design of a thermostable xylanase
Canale, S.; Galpern, E.; Ferreiro, D.U.; Campos, E.; Craig P.O.

EP-13
Molecular insight of cellulose degradation by the phototrophic green alga *Scenedesmus quadricauda*
Velazquez, M.B.; Busi, M.V.; Gomez-Casati, D.F.; Chitralekha, N.D.; Barchiesi, J.

EP-15
Mining an intertidal sediment metagenome for fucanases for the production of oligosaccharides from brown algae fucoidans
González, J. A.; Ponce, A.; Stortz, C.; Lozada, M.; Dionisi, H.

EP-17
Marine arabinofuranosidases: analysis of GH51 homolog sequences from a metagenomic dataset of an extreme environment
Dionisi, H.M.; Campos, E.

Abstracts

EP-15

Mining an intertidal sediment metagenome for fucanases for the production of oligosaccharides from brown algae fucoidans

González, J. A. (1); Ponce, A. (2); Stortz, C. (2); Lozada, M. (3); Dionisi, H. (1)

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email: 9gonzalezj@gmail.com

Fucoidans are a sulfated polysaccharides present in the cell wall matrix of brown algae. These polysaccharides are generally composed of a backbone of α-L-fucose residues, but they are very diverse in terms of monosaccharide composition, sulfate content and linkage patterns, i.e. α-(1,3) or α-(1,3)/α-(1,4). Fucoidans have a variety of biological activities beneficial for human health, such as immune-modulatory, antiviral, anticoagulant, antitumor, antithrombosis and antioxidant. However, the low absorption of these complex carbohydrates and their low bioavailability limit their health benefits besides their prebiotic effect. Endo-acting enzymes called fucanases depolymerize these polysaccharides, generating bioactive fuco-oligosaccharides with various biotechnological applications. So far, only two CAZy families have been described: GH107 (endo-α-1,4-L-fucanase) and GH168 (endo-α-1,3-L-fucanase). In this work, we mined putative fucanase sequences in a metagenomic dataset from intertidal sediments of Ushuaia Bay (Tierra del Fuego Island, Argentina) exposed to brown algae detritus, and selected sequences for heterologous expression and characterization. Using a series of HMMs specific for these families, we identified eight sequences homologous to the GH107 family and 32 sequences related to members of GH168 family. The relative abundance of these genes in the metagenome was one every 17,000 sequences, although probable only part of the diversity of these enzymes is currently known. Overall, 26 sequences shared low identity values (<40 % at protein level), while 5 sequences had high identity values (>80 %) with reference sequences of the database. One of the scaffold contained both GH107 and GH168 homologs, and often the genomic context of the identified sequences contained genes potentially related to fucoidan degradation, including α-fucosidases (exo-acting) and sulfatases. These results provide further evidence of the predicted function of the identified sequences. In both families, the most abundant taxonomic assignment of the scaffolds containing the identified sequences was the Planctomycetes phylum (35 % of the sequences), for which fucanase enzymes have not yet been characterized. The second most abundant taxonomic assignment was the Terrabacteria group, followed by Bacteroidetes and Proteobacteria phyla. Besides the catalytic module, domains identified in these sequences include a pectin lyase fold, carbohydrate-binding module, beta helix and domains belonging to other GH families (GH10, GH13, GH15, GH31 and GH29). Four sequences related to the GH107 family were selected for heterologous expression in *Escherichia coli*, three probably from members of the Planctomycetes phylum and one from the Bacteroidetes phylum. Low temperatures during expression were needed for reaching high levels of protein expression in the soluble fraction. The enzymes were purified and characterized, and fucanase activity was evaluated by carbohydrate-polyacrylamide gel electrophoresis (C-PAGE). The assessed substrates were fucoidans extracted from four brown algae species of the Patagonian coast: *Macrocystis pyrifera*, *Undaria pinnatifida*, *Scytosiphon lomentaria* and *Adenocystis utricularis*. Among the four expressed genes, #113643 (potentially from a Planctomycetes) presented a high activity towards *M. pyrifera* fucoidan, with degradation products observed from a 30 s incubation time at 25 °C, faster than previously characterized members of the GH107 family. The enzyme was active in a wide range of temperatures (5 - 45 °C), salinities (9.5 – 861 mM NaCl) and pH (4.5 – 9). The best template for the 3D modeling of #113643 was the structure of the fucanase P5AFcnA from *Psychromonas* sp. SW5A (21 % identity and 70 % coverage), enzyme that is able to degrade the same substrate. The structure of *M. pyrifera* fucoidan is poorly defined, and very limited information is available on how the active-site topology relates to fucanase substrate specificity, in particular because substrate recognition depend not only on the glycosidic linkages but also on modifications such as sulfation patterns. Further work will include the use of substrates purified from other brown algae species, the heterologous expression and characterization of member of the GH168 family and structural analyses of the degradation products. As a result of this study, novel fucanase enzymes will be available to produce fuco-oligosaccharides from brown algae species of Patagonia, with nutraceutical, cosmeceutical and pharmaceutical applications.

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