



**Biotech**  
FRANCE 2018

# Biotech France 2018

International Conference and Exhibition

27th - 29th June, 2018

Paris – France

## Book of Abstracts

Organizer



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Conferences & Exhibitions

# Biotech France 2018 Conference Program

<b>June 26, 2018</b>		
15:00 - 17:00	Registration	Registration Area
<b>June 27, 2018</b>		
<b>Biotech France 2018 - Session I: Biochemical / Bioprocess Engineering / Pharmaceutical / Medical Biotechnology</b>		
<b>Conference Room 508</b>		
<b>Session's Chairs:</b> <b>Dr. Yvonne van der Meer, Maastricht University, The Netherlands</b> <b>Prof. Mathieu Sperandio, INSA Toulouse, France</b>		
08:30 - 12:00	Conferences Registration	Registration Area
10:00 - 10:30	Maximizing the atom efficiency of redox reactions: Exploiting the exquisite selectivity of oxidoreductases <b>F. Mutti</b>	Prof. <b>Francesco Mutti</b> , University of Amsterdam, <b>The Netherlands</b>
10:30 - 11:00	Coffee Break / Posters Session I	Coffee Break Area
11:00 - 11:30	Development of Biotechnological Approach for the Production of Novel Nutraceuticals and Natural Bio-ingredients and Their Encapsulation <b>S. Kermasha</b>	Prof. <b>Selim Kermasha</b> , McGill University, <b>Canada</b>
11:30 - 11:45	On the way towards cofactor supply via flow Biocatalysis <b>F. Busch</b> , M. Schürmann and R. Kourist	<b>Mr. Florian Busch</b> , InnoSyn B.V., Geleen, <b>The Netherlands</b>
11:45 - 12:00	Endophytic fungi as robust biocatalysts to oxyfunctionalize hydrocarbon terpenes F.Cecati and <b>M.Kurina-Sanz</b>	<b>Dr. Marcela Kurina-Sanz</b> , San Luis National University, <b>Argentina</b>
12:00 - 12:15	The use of Laccase in a biotechnological approach for the degradation of aflatoxins <b>S. Borgomano</b> , M. Lacroix and S. Kermasha	<b>Dr. Sabrina Borgomano</b> , INRS-Institute Armand- Frappier, <b>Canada</b>
12:30 - 14:00	Lunch Break	Restaurant (Rue Basse)

<b>June 27, 2018</b>		
<b>Biotech France 2018 - Session II: Environmental Biotechnology - Green Economy</b>		
<b>Conference Room 508</b>		
<b>Session's Chairs:</b> <b>Prof. Pierre Monsan, INSA Toulouse, France</b> <b>Dr. Yvonne van der Meer, Maastricht University, The Netherlands</b> <b>Prof. Ica Manas-Zloczower, Case Western Reserve University, Cleveland, USA</b>		
14:00 - 14:30	Refactoring carbon metabolism of microbial systems for added value production from renewable carbon source <b>J-M. Francois</b>	Prof. <b>Jean-Marie Francois</b> , INSA de Toulouse, <b>France</b>
14:30 - 14:45	Biotechnology Innovation Observatory <b>R.F. de Brito</b> , M.Shintaku and D.J. Macedo	<b>Dr. Ronnie Fagundes de Brito</b> , Brazilian Institute of Information in Science and Technology, <b>Brazil</b>
14:45 - 15:00	Statistical optimization of chitin extraction from mushroom wastes by lactic acid fermentation in a stirred tank bioreactor <b>N. Patra</b> and N. Abu-Ghannam	<b>Dr. Nivedita Patra</b> , Institute of Technology Rourkela, <b>India</b>
15:00 - 15:30	From BioSciences to BioProduction: The Pre-industrial Demonstrator TWB. <b>P. Monsan</b>	Prof. <b>Pierre Monsan</b> , INSA Toulouse, <b>France</b>
15:30 - 16:00	Environmental impact of bio-based products <b>Y. Van der Meer</b>	<b>Dr. Yvonne van der Meer</b> , Maastricht University, <b>The Netherlands</b>

16:00 - 16:30	Coffee Break / Posters Session I	Coffee Break Area
16:30 - 17:00	Engineering structural composites using bio-based materials L. Yue, A. Patel, Y. Qiang and <b>I. Manas-Zloczower</b>	<b>Prof. Ica Manas-Zloczower</b> , Case Western Reserve University, Cleveland, <b>USA</b>
17:00 - 17:30	Microbial resource management for environmental biotechnology <b>M. Sperandio</b>	<b>Prof. Mathieu Sperandio</b> , INSA Toulouse, <b>France</b>
17:30 - 17:45	Production and use of microbial biomass helping sustainability in fish production chain E. H. G. Ponsano, T. L. M. Grassi, E. F. E. Santo, L. K. F. Lima and <b>R. C. Pereira</b>	<b>Prof. Elisa Ponsano</b> , Estadual Paulista University, <b>Brazil</b>
17:45 - 18:00	Pesticides in the Environment and Mitigation Technologies <b>M. C. Diez</b> , M. Levío and F. Gallardo	<b>Dr. María Cristina Diez</b> , University of La Frontera, <b>Chile</b>

<b>June 27, 2018</b>		
<b>Biotech France 2018 - Session III: Pharmaceutical / Medical Biotechnology</b>		
18:00 - 18:15	Implementation of bacterial expression systems for the detection of L-asparaginase activity in droplet-based microfluidics <b>M. Morvan</b> , A. Vigne, J. Lopez Morales, C.S. Karamitros, M.Konrad and J.C. Baret	<b>Mr. Mickaël Morvan</b> , Bordeaux University, <b>France</b>
18:15 - 18:30	Targeting Cancer at the Nuclear Pore <b>A.S. Azmi</b>	<b>Dr. Asfar Azmi</b> , Wayne State University, <b>USA</b>
18:30 - 18:45	Reliability of 2D Kinematic Analysis of Sagittal and Frontal Plane Motion During Running in Individuals with Patellofemoral Pain <b>L. Cedin</b> , T.C. Rosa, B.O. Peixoto and D.H. Kamonseki	<b>Prof. Luísa Cedin</b> , Paulista University, <b>Brazil</b>
18:45 - 19:00	Genetic transformation of Physcomitrella patens to overexpress Pp-miR536 <b>C.X. Ordoñez</b> , S.R. Meléndez, A.A. Becerra, M. Á. Villalobos López, F.F.R.Cárdenas and J.G. Jorge	<b>Ms. Carmina Xicohtencatl Ordoñez</b> , National Polytechnic Institute, <b>Mexico</b>

<b>June 28, 2018</b>		
<b>Nanotech / Biotech Joint Plenary session II</b>		
<b>Amphitheatre H</b>		
<b>Session's Chairs:</b>		
<b>Prof. Francesco Mutti, University of Amsterdam, The Netherlands</b>		
<b>Dr. Olivier Sandre, Institut Polytechnique de Bordeaux, France</b>		
<b>Dr. Jean-Olivier Durand, Institut Charles Gerhardt Montpellier, France</b>		
<b>Dr. Winnie Edith Svendsen, Technical University of Denmark, Denmark</b>		
08:30 - 09:00	From antibodies to Metal Organic Frameworks: a Full Set of Enveloppes for metal cofactors in order to build up new artificial metalloenzymes <b>J-P.Mahy</b> , W. Ghattas, F. Avenier and R. Ricoux	<b>Prof. Jean-Pierre Mahy</b> , Paris-Sud University, <b>France</b>
09:00 - 09:30	Expanding and exploring natural sequence space – from protein engineering to chemo-enzymatic cascade reactions C. Mügge, Á. Gomez-Baraibar and <b>R. Kourist</b>	<b>Prof. Robert Kourist</b> , Ruhr University Bochum, Germany/ Graz University of Technology, <b>Austria</b>
09:30 - 10:00	Design and Evolution of New Biocatalysts for Organic Synthesis <b>N. J. Turner</b>	<b>Prof. Nicholas Turner</b> , The University of Manchester, <b>UK</b>
10:00 - 10:30	Practical biocatalytic solutions for the design of chemoenzymatic and multienzymatic concurrent processes <b>V. Gotor-Fernández</b>	<b>Prof. Vicente Gotor- Fernandez</b> , University of Oviedo, <b>Spain</b>
10:30 - 11:00	Coffee Break / Posters Session II	Coffee Break Area

11:00 - 11:30	Nanomedicines for the treatment of cancer and neurological diseases <b>P. Couvreur</b>	<b>Prof. Patrick Couvreur,</b> UMR CNRS 8612, <b>France</b>
11:30 - 12:00	Nanotechnology against viral diseases <b>F. Stellacci</b>	<b>Prof. Francesco Stellacci,</b> Ecole Polytechnique de Lausanne, <b>Switzerland</b>
12:00 - 12:45	Applications of Artificial Intelligence in Biotech and Nanotech research <b>M. Cristovao</b>	<b>Dr. Michele Cristovao,</b> Springer Nature, <b>Germany</b>
12:30 - 14:00	<b>Lunch Break</b>	<b>Restaurant (Rue Basse)</b>

**June 28, 2018 – 13:45 – 13:55**

**Conference Group Photo**

**At the conference registration desk (located at Rue Haute)**

**All conference participants are requested to be present for the Conference Group Photo**

<b>June 28, 2018</b>		
<b>Biotech / Nanotech Joint Session II.A: Nanotechnology for life science</b>		
<b>Conference Room 109/110</b>		
<b>Session's Chairs:</b> <b>Dr. Stéphane Mornet, Bordeaux University, France</b> <b>Prof. Rui Silva, University of Aveiro, Portugal</b> <b>Prof. Giulio Caracciolo, Sapienza University of Rome, Italy</b>		
14:00 - 14:30	Brain Structure and Function Combine to Create the Characteristics of a Bio-Metamaterial <b>S.D.Morgera</b>	<b>Prof. Salvatore Domenic Morgera,</b> University of South Florida, <b>USA</b>
14:30 - 15:00	Nanostructures for biological and environmental applications <b>W.E. Svendsen</b>	<b>Dr. Winnie Edith Svendsen,</b> Technical University of Denmark, <b>Denmark</b>
15:00 - 15:30	Clickable' Recombinant Spider Silk and its Healthcare Applications <b>N.R. Thomas,</b> D. Harvey, R. Earlam, P. Bardelang, S.L. Goodacre and A. Cockayne	<b>Prof Neil R. Thomas,</b> University of Nottingham, <b>UK</b>
15:30 - 16:00	Surface nanoengineering of intravenously administered inorganic nanoparticles L. Adumeau, C. Vecco-Garda, G. Clofent-Sanchez, C. Genevois, F. Couillaud and <b>S.Mornet</b>	<b>Dr. Stéphane Mornet,</b> ICMCB (UMR 5026 CNRS - Bordeaux University - Bordeaux INP), <b>France</b>
16:00 - 16:30	<b>Coffee Break / Posters Session II</b>	<b>Coffee Break Area</b>
16:30 - 16:45	Vitamin A palmitate-loaded NLC for cosmetic application <b>S. AlZahabi</b> and A.R. Ramadan	<b>Ms. Sham AlZahabi,</b> American Uni. Cairo, <b>Egypt</b>

**June 28, 2018**

**Biotech / Nanotech Joint Session II.B: Nanomedicine- Bioimaging**

**Conference Room 111/112**

**Session's Chairs:**

**Dr. Valeria Grazú, University de Zaragoza, Spain**  
**Prof. Wolfgang Ensinger, Technische Universitaet Darmstadt, Germany**  
**Prof. Neil R. Thomas, University of Nottingham, UK**

14:00 - 14:30	The iNAPO project: Biomimetic ion conducting polymer nanopores for bio-molecular and chemical sensing <b>W. Ensinger,</b> M. Biesalski, G. Buntkowsky, K. Hamacher, B. Laube, H. F. Schlaak, G. Thiel, Ch. Trautmann, N. van der Vegt and M. Vogel	<b>Prof. Wolfgang Ensinger,</b> Technische Universitaet Darmstadt, <b>Germany</b>
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14:30 - 14:45	Confocal Laser Endomicroscopy Guided Photothermal/Photodynamic Therapy of Pancreatic Cancer <b>H. Li</b> , K. Yang and Y. Cheng	<b>Dr. Hui Li</b> , Shanghai Jiaotong University, <b>China</b>
14:45 - 15:00	Dual Metallofluorescent Nanoparticles for live cells assays <b>A. Delgado-Gonzalez</b> , E. Garcia-Fernandez, T. Valero, M.V. Cano-Cortes, M.J. Ruedas-Rama, A. Unciti-Broceta, A. Orte, R.M. Sanchez-Martin and J.J. Diaz-Mochon	<b>Mr. Antonio Delgado-Gonzalez</b> , University of Granada, <b>Spain</b>
15:00 - 15:15	Design of Polyelectrolyte Microcapsules Encoded with Excitonic Nanoparticles and Prospects of their Applications as Novel Bio-imaging and Theranostic Tools <b>G. Nifontova</b> , M. Zvaigzne, M. Baryshnikova, E. Korostylev, F. Ramos-Gomes, F. Alves, I. Nabiev and A. Sukhanova	<b>Dr. Galina Nifontova</b> , National Research Nuclear University MEPhI-Moscow, <b>Russia</b>
15:15 - 15:30	Development of new theranostic platforms based on carbon dots <b>M. Claudel</b> , J. Fan, F. Pons and L. Lebeau.	<b>Mr. Mickaël Claudel</b> , University of Strasbourg, <b>France</b>
15:30 - 15:45	Reduction of methemoglobin to oxyhemoglobin under influence of nanoparticles of perfluorocarbon emulsion and cytoflavin. <b>E.A. Manchenko</b> , E.K. Kozlova, A.M. Chernysh and V.A. Sergunova	<b>Mrs. Ekaterina Manchenko</b> V.A.Negovsky Scientific Research Institute of General Reanimatology- Moscow, <b>Russia</b>
15:45 - 16:00	Nanostructure as biomarkers for the diagnosis of donor blood during long-term storage. <b>V.A. Sergunova</b> , E.K. Kozlova, A.M. Chernysh and E.A. Manchenko	<b>Mrs. Victoria Sergunova</b> , V.A. Negovsky Scientific Research Institute of General Reanimatology- Moscow, <b>Russia</b>
<b>16:00 - 16:30 Coffee Break / Posters Session II Coffee Break Area</b>		
16:30 - 16:45	Novel Approach to Flow Label-Free Multiplex Biosensing via Photonic Crystal Surface Wave Detection Technique <b>I.O. Petrova</b> , V.N. Konopsky, Nabiev and A. Sukhanova	<b>Dr. Irina Petrova</b> , National Research Nuclear University MEPhI-Moscow, <b>Russia</b>
16:45 - 17:00	Feasibility of magnetic nanoparticles encapsulated inside carbon nanotubes for hyperthermia <b>R. Ghunaim</b> , S. Hampel, R. Klingeler and B. Büchner	<b>Ms. Rasha Ghunaim</b> , Leibniz Institute for Solid State and Material Research Dresden, <b>Germany</b>
17:00 - 17:15	Magnetic Nanozyme-Linked Immunosorbent Assay for Ultrasensitive Influenza A Virus Detection S. Oh, J. Kim, V.Tan Tran, D. Kyu Lee and <b>J. Lee</b>	<b>Prof. Jaebeom Lee</b> , Pusan National University, <b>Rep. of Korea</b>
17:15 - 17:30	Targeting and Killing of Leukemic Cells with Magnetic Nanowires <b>N. Alsharif</b> , J. Merzaban, T. Ravasi and J. Kosel	<b>Ms. Nouf Alsharif</b> , KAUST, <b>Saudi Arabia</b>
17:30 - 17:45	Bare Magnetic Nanoparticles for Protein Recognition <b>S. Schwaminger</b> , S. Blank-Shim, P. Anand, M. Borkowska- Panek, K. Fink, P. Fraga-García, W. Wenzel and S. Berensmeier	<b>Mr. Sebastian Schwaminger</b> , Technical Univ. of Munich, <b>Germany</b>

<b>June 28, 2018</b>		
<b>Biotech / Nanotech Joint Focused Session on Nanotechnology for drug and gene delivery</b>		
<b>Conference Room 561</b>		
<b>Session's Chairs:</b>		
<b>Dr. Olivier Sandre, Institut Polytechnique de Bordeaux, LCPO, France</b>		
<b>Dr. Sonia Trigueros, University of Oxford, UK</b>		
14:00 - 14:30	Magnetic Iron Oxide Nanoparticles Grafted by a Thermosensitive Peptide Brush: Uptake by Tumor Cells and Cytotoxicity by Magnetic Hyperthermia <b>O. Sandre</b>	<b>Dr. Olivier Sandre</b> , Institut Polytechnique de Bordeaux – LCPO, <b>France</b>
14:30 - 15:00	Nano-Delivery Overcoming the major challenges in Drug and Gene delivey <b>S. Trigueros</b>	<b>Dr. Sonia Trigueros</b> , University of Oxford, <b>UK</b>

15:00 - 15:30	Mesoporous silica, periodic mesoporous organosilica, and mesoporous silicon nanoparticles for drug delivery and two-photon Photodynamic Therapy <b>J-O. Durand</b>	<b>Dr. Jean-Olivier Durand</b> Institut Charles Gerhardt Montpellier, France
15:30 - 16:00	Nanotherapeutics for Targeted Elastic Matrix Regenerative Repair in Vascular Disorders A. Camardo, S.Carney, N. Sharma and <b>A.Ramamurthi</b>	<b>Dr. Anand Ramamurthi</b> , Cleveland Clinic, USA
16:00 - 16:30	<b>Coffee Break / Posters Session II</b>	
		<b>Coffee Break Area</b>
16:30 - 16:45	Challenges on the development of nanotherapeutics: biophysical studies to guide formulation development E.Fernandes, T.B. Soares, H.Gonçalves and <b>M. Lúcio</b>	<b>Dr. Marlene Lúcio</b> University of Minho, Portugal
16:45 - 17:00	Biophysical characterization based on biomimetic nanosystems/drug interactions: a new strategy for a rational drug design process <b>E. Fernandes</b> , S. Bernstorff and M. Lúcio	<b>Mrs. Eduarda Fernandes</b> , University of Minho, Portugal
17:00 - 17:15	Diclofenac interaction with lipid nanosystems as membrane models: a bio-physical assessment of in vitro profiling <b>T.B Soares</b> , E. Fernandes, S. Bernstorff and M. Lúcio	<b>Ms. Telma Bezerra Soares</b> , University of Minho, Portugal
17:15 - 17:30	Novel oxide nanomaterials for drug delivery through the blood-brain-barrier <b>W. Lipinski</b> , M.M. Godlewski, J. Kaszewski, Z. Gajewski and M. Godlewski	<b>Mr. Waldemar Lipinski</b> , Faculty of Veterinary Medicine- Warsaw, Poland
17:30 - 17:45	SiO <sub>2</sub> nanoparticles as a vehicle for delivery of nucleoside triphos-phate analogues into cells <b>S. Vasilyeva</b> , A. Shtil, I. Grin and D. Stetsenko	<b>Dr. Svetlana Vasilyeva</b> , Siberian Branch of the Russian Academy of Sciences, Russia
17:45 - 18:00	Synthesis of PHA nanoparticles for drug delivery: optimizing the size distribution via the effect of the surfactant <b>V. Amstutz</b> , N. Hanik and M. Zinn	<b>Dr. Véronique Amstutz</b> , University of Applied Sciences and Arts Western Switzerland, Switzerland
18:00 - 18:15	A report on synthesis of NIR light responsive nanoparticles-in-microparticles by a double emulsion method: Photothermal and drug delivery use in future <b>M. Dhanka</b> , D. S Chauhan and R. Srivastava	<b>Mr. Mukesh Dhanka</b> , Indian Institute of Technology Bombay, India

<b>June 29, 2018</b>		
<b>Biotech/ Nanotech Joint Session III.C: NanoMedicine / Nanosafety</b>		
<b>Conference Room 558</b>		
<b>Session's Chairs:</b>		
<b>Dr. Jean-Olivier Durand, Institut Charles Gerhardt Montpellier, France</b>		
<b>Dr. Sonia Trigueros, University of Oxford, UK</b>		
09:00 - 09:30	Short, long term fate and biodegradation of IONPs in vivo <b>V. Grazú</b> and J. M. de la Fuente	<b>Dr. Valeria Grazú</b> , University de Zaragoza and CIBER-BBN, Spain
09:30 - 09:45	Nanoparticle delivery of drugs for Tuberculosis <b>I.L. Batalha</b> , A. Bernut, R.A. Floto and M.E. Welland	<b>Dr. Iris Batalha</b> , University of Cambridge, UK
09:45 - 10:00	Synthesis of Gold Nanovehicles for Con-trolled Drug Delivery Applications <b>R. Lopes Rodrigues</b> , F.Xie, A. Porter and M. Ryan	<b>Ms. Rosalia L. Rodrigues</b> , Imperial College London, UK
10:00 - 10:30	<b>Coffee Break</b>	
		<b>Coffee Break Area</b>
10:30- 11:00	Nanopattern improves chondrogenesis for cartilage regeneration. I.Casanellas, A. Lagunas, I. Tsintzou, Y. Vida, D. Collado, E. Pérez-Inestrosa, C. Rodríguez Pereira, J. Magalhaes and <b>J.Samitier</b>	<b>Prof. Josep Samitier Martí</b> , Institute for Bioengineering of Catalonia (IBEC), Spain

11:00 - 11:15	Characterization of the interaction of graphene oxide with the mammalian sperm membrane <b>J.Simões</b> , M.Ramal Sanchez, R. Zappacosta M. Ciulla, A.Di Stefano, A. Fontana, P. Lanuti, E.Ercolino, M. Marchisio, G. Capacchietti, L. Valbonetti, N. Bernabò and B. Barboni	<b>Ms Juliana Simões</b> , University of Teramo, <b>Italy</b>
11:15 - 11:30	A Potential Approach to Assess and Control the Potential Risks Related to Nanomaterials <b>C. Schimpel</b> , S. Resch and A. Falk	<b>Ms. Christa Schimpel</b> , BioNanoNet, <b>Austria</b>
11:30 - 11:45	Tuball™ Single wall Carbon Nanotubes: Health, Safety & Environmental issues <b>G. Van Kerckhove</b>	<b>Mr. Gunther Van Kerckhove</b> , OCSiA Europe Sarl, <b>Luxembourg</b>
11:45 - 12:00	Review of human health risk assessment models considering their input requirements and applicability during nanomaterial product development results from the EU H2020 'CALIBRATE' project <b>T. Oosterwijk</b> , R. Franken, M. Heringa, I. Rodriguez, A. Saämanen, T. Kanerva, M. Dal Maso, M. Poikkimaki, K.A. Jenssen, C. de Jong-Rubingh, R. Stierum and W. Fransman	<b>Mr. Thies Oosterwijk</b> ,TNO, Risk Assessment of Products In Development, <b>The Netherlands</b>

**June 29, 2018**

**Biotech / Nanotech Joint Session III.D: Nanomaterials for food applications**

**Conference Room 461**

**Session's Chairs:**

**Dr. Clara Silvestre, ICTP/CNR, Naples, Italy**

**Dr. Giovanna G. Buonocore, ICTP/CNR, Naples, Italy**

09:00 - 09:30	Nanotechnology in the food packaging sector: recent applications and future trends <b>A.Sanches Silva</b>	<b>Dr. Ana Sanches Silva</b> , National Institute of Agrarian and Veterinary Research, <b>Portugal</b>
09:30 - 10:00	Active biopolymer film or coating for food packaging application: structure-properties relationship and shelf life extension <b>E. Torrieri</b>	<b>Prof. Elena Torrieri</b> , University of Naples Federico II, <b>Italy</b>
10:00 - 10:30	<b>Coffee Break</b>	
		<b>Coffee Break Area</b>
10:30 - 10:45	PAA.PVA-PAMAM bio-nanocomposite films incorporating thymol for food packaging <b>G. Amariei</b> , K. Boltes, I. Iriepa, I. Moraleda, P. Letón and R. Rosal	<b>Ms. Georgiana Amariei</b> University of Alcalá, <b>Spain</b>
10:45 - 11:00	A novel antibacterial strategy based on oxide nanoparticles for medical and food-related polygraphy <b>J. Cymerys-Bulenda</b> , R. Pietuszka, A. Słońska-Zielonka, S. Gierałowska, B.S. Witkowski, Z. Gajewski, M. M. Godlewski and M. Godlewski	<b>Dr. Joanna Cymerys-Bulenda</b> , Warsaw University of Life Sciences – SGGW, <b>Poland</b>

**Posters Session I: June 27, 2018  
Biotech France 2018**

**Exhibition and Posters Hall**

<b>N.</b>	<b>Title</b>	<b>Author/Affiliation/Country</b>
<b>1</b>	Bioinformatic identification method of evolutionary ranges for functional small peptides on genomes <b>H. Takahashi</b> , N. Hayashi, A. Takahashi and H. Onouchi	<b>Dr. Hiro Takahashi</b> , Kanazawa University, <b>Japan</b>
<b>2</b>	Enhancing Cas9 nuclease expression in Escherichia coli using defined media <b>G.P. Carmignotto</b> and A.R. Azzoni	<b>Ms. Gabriela Carmignotto</b> , University of Sao Paulo, <b>Brazil</b>
<b>3</b>	Novel Bioreactor System for Continuous IgG Production <b>M.Hagihara</b> , S. Ohya, T.Harada, S.Yamada	<b>Mr. Masahiko Hagihara</b> UBE Industries, Ltd, <b>Japan</b>
<b>4</b>	Isolation and selection of microorganisms capable of tolerating different pesticides for the construction of a microbial consortium <b>R.A. Saldaña</b> , Á.E. Absalón and D.V. Cortés-Espinosa	<b>Ms. Rosa Arenas Saldaña</b> , National Polytechnic Institute, <b>Mexico</b>
<b>5</b>	Dendron-Grafted Polylysine-Based Dual-Modal Nanoprobe for Ultra-Early Diagnosis of Pancreatic Precancerosis via Targeting a Urokinase-Type Plasminogen Activator Receptor H. Li, K. Yang, and <b>Y. Cheng</b>	<b>Dr. Cheng Yingsheng</b> , Shanghai Jiaotong University, <b>China</b>
<b>6</b>	Niemann-Pick Type C2 Protein Inhibits PBGF-BB-Induced Hepatic Stellate Cells Proliferation <b>Y.H. Wang</b> , Y. C. Twu and Y.J. Liao	<b>Ms. Yuan His Wang</b> , National Yang-Ming University, <b>Taiwan</b> .
<b>7</b>	Selenate Reduction to Elemental Selenium by Shigella sonnei strain SE6-1 and Its Potential Mechanism Elucidated by Genome Sequence A. Cho, H. Lee and <b>J-H. Lee</b>	<b>Dr. Ji-Hoon Lee</b> , Chonbuk National University, <b>Rep.of Korea</b>
<b>8</b>	Improved shoot regeneration, salinity tolerance and reduced fungal susceptibility in transgenic tobacco constitutively expressing PR-10a gene <b>M.Dabi</b> , P. Agarwal and P.K Agarwala	<b>Ms. Mitali Dabi</b> , CEA Saclay, <b>France</b>

**Posters Session II: June 28, 2018**

**Biotech/ Nanotech Joint session on NanoBioMedicine / Nanosafety**

**Exhibition and Posters Hall**

<b>1</b>	Conductance measurements in Laponite-stabilized internally self-assembled particles in water. <b>C. Barth</b> , T. Dégousée, S. Gallanti and F. Muller	<b>Dr. Céline Barth</b> , ECE-Paris Engineering School, <b>France</b>
<b>2</b>	Capture and growth of cells on the ligand modified polystyrene chips coated with agarose and agarose/gelatin <b>M.K. Lee</b> and J. Jeong	<b>Dr. Myung Kyu Lee</b> , Korea Research Institute of Bioscience and Biotechnology, <b>Rep. of Korea</b>
<b>3</b>	Selective Claudin-4 Targeting of Clostridium Perfringens Enterotoxin (CPE)-conjugated Poly-sialic acid Nanoparticles for effective pancreatic cancer therapy M.K. Shim, I.K. Cho, K. Kim and <b>J-H. Kim</b>	<b>Prof. Jong-Ho Kim</b> , Kyung Hee University, <b>Rep. of Korea</b>
<b>4</b>	Silver-Polyvinyl Pyrrolidone (Ag-PVP) Nanoparticles Exhibit Antibacterial Activity against Chlamydia muridarum in Mouse J774 Macrophages S.Dixit, S. R. Singh and <b>V.A. Dennis</b>	<b>Dr. Vida A. Dennis</b> , Alabama State University, <b>USA</b>
<b>5</b>	Numerical optimization of the carboplatin encapsulation into Boron Nitride nanotubes <b>J. Bentin</b> and F. Picaud	<b>Mr. Jeremy Bentin</b> , University of Bourgogne-Franche-Comté, <b>France</b>
<b>6</b>	A new neural-cell specific peptide for targeted delivery of drug-loaded nanoparticles <b>R. Huey</b> , D. Rathbone, P. McCarron and S. Hawthorne	<b>Ms. Rachel Huey</b> , Ulster University, <b>UK</b>
<b>7</b>	Cationized Polymer (dCatAlb) Encrusted Nanoformulation enhance the chemotherapeutic activity of Doxorubicin V. Jhonson, <b>N. Raval</b> , P.Gondaliya, V.Tambe, K.Kalia and R. Tekade	<b>Ms. Nidhikumari Raval</b> , National Institute of Pharmaceutical Education and Research, <b>India</b>

8	Self-assembled Polymeric Nanoparticles for Targeting Mitochondrial Complex II K. Kwon, G. Battogtokh, Y.-Y. Cho, J. Y. Lee, H. S. Lee and <b>H. C. Kang</b>	<b>Prof. Han Chang Kang</b> , The Catholic University of Korea, <b>Rep. of Korea</b>
9	Customized D2B-gold coated Nanoparticles: promising therapeutic agents against prostate cancer. M. Sarkis, G. Minassian, H. Naim, G. Fracasso, J.D. Holmes, K. Rahme and <b>E.Ghanem</b>	<b>Dr. Esther Ghanem</b> , Notre Dame University, <b>Lebanon</b>
10	Exploitation of the liposome-biomolecular corona for early detection of pancreatic cancer D. Pozzi, L. Digiacomo, S. Palchetti, F. Giulimondi, M. Cartillone, C. Cascone, R. Coppola, D. Caputo and <b>G. Caracciolo</b>	<b>Prof. Giulio Caracciolo</b> , Sapienza University of Rome, <b>Italy</b>
11	Enhanced Gene Transfection by Multifunctional Properties of Polymeric Vitamins H. Cho, J. Y. Lee, Y.-Y. Cho, <b>H. S. Lee</b> and H. C. Kang	<b>Prof. Hye Suk Lee</b> , The Catholic University of Korea, <b>Rep. of Korea</b>
12	Preparation and physicochemical characterization of nanostructured iron(III) hydroxyphosphates as potential vaccine adjuvants <b>N. Angelova</b> and G. Yordanov	<b>Ms. Nadezhda Angelova</b> , Sofia University, <b>Bulgaria</b>
13	Gold nanoparticle-based colorimetric immunosensor for estradiol <b>A. Minopoli</b> , B. Della Ventura, C. Schiattarella, N. Sakač and R. Velotta	<b>Mr. Antonio Minopoli</b> , University of Naples "Federico II", <b>Italy</b>
14	Big Instrument- and Chaotropic Detergent-Free Assay for Ultra-sensitive Biomolecule Nucleic Acid Isolation and Detection Via Binary Nanomaterial <b>H. F. Liu</b> , F. Zhao, E. Y. Lee and Y. Shin	<b>Ms. Huifang Liu</b> , University of Ulsan, <b>Rep. of Korea</b>
15	Fabrication of Highly Sensitive Ammonia Sensor: Potential Use for Diagnosis Purpose <b>T.N. Ly</b> and S. Park	<b>Mr. Tan Nhiem Ly</b> , Dongguk University, <b>Rep. of Korea</b>
16	Rapid and Sensitive Detection of pathogen diagnosis based on Microfluidic Enrichment with a Label-free Nanobiosensing Platform <b>T.N.T. Dao</b> , J. Yoon, C. Eun Jin, B. Koo, E. Yeong Lee,, K. Han, T.Y. Lee and Y. Shin	<b>Ms. Nguyen Dao</b> , Ulsan University, <b>Rep.of Korea</b>
17	Development of X-shaped DNA as an immune adjuvant for the cancer immunotherapy through dual activation of TLR9 and inflammasomes J.E. Koo, H.E. Lee, S.H. Eom, H.C. Kang, Y-Y. Cho, H.S. Lee and <b>J.Y. Lee</b>	<b>Dr. Joo Lee</b> , The Catholic University of Korea, <b>Rep.of Korea</b>

**Biotech France 2018 - Session I:  
Biochemical / Bioprocess  
Engineering / Pharmaceutical /  
Medical Biotechnology**

# Maximizing the atom efficiency of redox reactions: Exploiting the exquisite selectivity of oxidoreductases

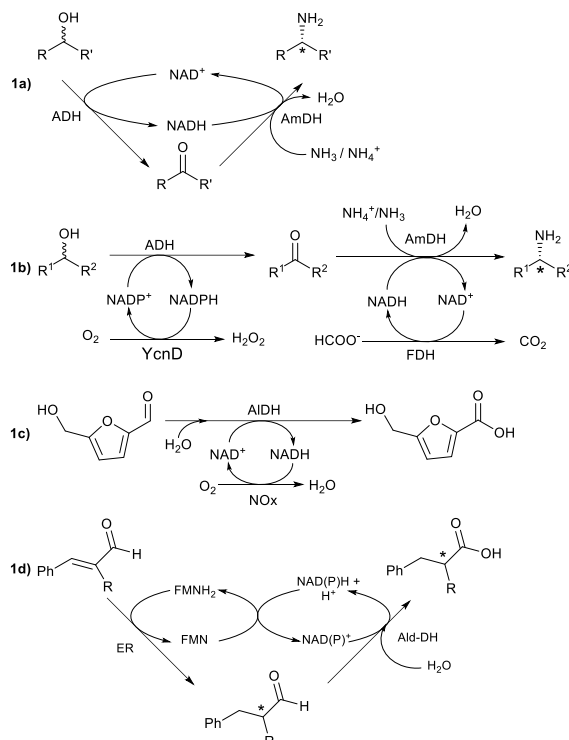
T. Knaus,<sup>1</sup> L. Cariati,<sup>1</sup> M. F. Masman,<sup>1</sup> V. Tseliou,<sup>1</sup> M. L. Corrado,<sup>1</sup> F. G. Mutti,<sup>1,\*</sup>

<sup>1</sup> University of Amsterdam, van't Hoff Institute for Molecular Sciences, HIMS-Biocat, Amsterdam, The Netherlands, \*e-mail: f.mutti@uva.nl

## Abstract:

Oxidoreductase enzymes enable the selective manipulation of functional groups with unrivalled efficiency. For instance, the exquisite selectivity of some dehydrogenases allowed us to develop new biocatalytic strategies for the synthesis of enantiopure amines or  $\alpha$ -substituted carboxylic acids. Figure 1a shows the conversion of alcohols into enantiopure primary amines through the combination of an alcohol dehydrogenase (ADH) with an amine dehydrogenase (AmDH). The internal recycling of redox equivalents maximizes the atom efficiency of the dual-enzyme system. Ammonia is the only reagent, whereas water is the only by-product. Another option (1b) consists of an orthogonal network in which the oxidative and the reductive step run concomitantly, but without any physical separation. Such a process profits from the divergent cofactor dependence of some dehydrogenases for NAD or NADP. Thus, the dehydrogenases employed in this biocatalytic network showed highly specificity for the type of cofactor and for the type of reaction, but also a high level of substrate promiscuity because structurally diverse alcohols could be aminated. Another study (1c) revealed the perfect chemoselectivity of some aldehyde dehydrogenases (AIDH). In fact, a set of more than fifty aldehydes were oxidized to carboxylic acids but leaving untouched any other oxidizable functional group. The superior selectivity of these AIDHs to distinguish between  $\alpha,\beta$  unsaturated and saturated aldehydes was exploited in the asymmetric conversion of  $\alpha$ -substituted  $\alpha,\beta$  unsaturated aldehydes into enantiopure carboxylic acids (1d). The internal recycling of the hydride maximizes the atom efficiency as observed in the case of the amination of alcohols (1a).

**Keywords:** redox reactions, oxidoreductases, selectivity, enzymatic cascades, dehydrogenases.



**Figure 1:** Redox biocatalytic cascades.

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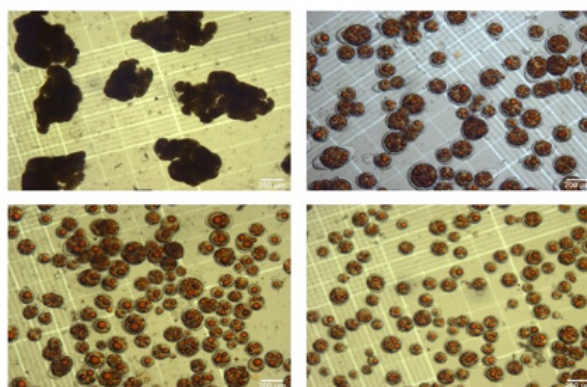
# Development of Biotechnological Approach for the Production of Novel Nutraceuticals and Natural Bio-ingredients and Their Encapsulation

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Ste-Anne de Bellevue, Qc, Canada

## Abstract:

There is a growing interest in the development of nutraceuticals as food supplements as well as natural bio-ingredients to fulfill the high consumer's demand for health-promoting food products. The numerous health benefits of the  $\omega$ -3 and  $\omega$ -6 polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) have been widely recognized in the modulation of risk of a variety of diseases. The incorporation of selected PUFAs into the diet is therefore of great importance. The human body cannot synthesize these PUFAs de novo and they can be obtained by a direct intake of selected food products, mainly vegetable and marine dietary sources. Nevertheless, the formulation of nutraceuticals with PUFAs cannot be done without simultaneous incorporation of an appropriate antioxidant. Although endogenous phenolic compounds are considered excellent sources of natural antioxidants, their low solubility in non-polar media reduces their effectiveness in fat and oil systems. The incorporation of phenolic acids into triacylglycerols by lipase-catalyzed transesterification could potentially result in novel structured phenolic lipids (PLs), with enhanced anti-oxidative, improved solubility and functional properties. Research work, carried out in our laboratory, has succeeded in the enzymatic synthesis of structured PLs in non-conventional media, using edible oils including flaxseed oil, fish oil and krill oil and selected phenolic acid models. In addition, a procedure for the encapsulation of PLs was developed and the encapsulated product was structurally characterized and its antioxidant potential as well as its stability were assessed. The structured PLs could not be used only as nutraceuticals but also as natural antioxidant ingredients in food industry



**Figure 1:** Microscopic images of encapsulated structured phenolic lipids (PLs).

## References:

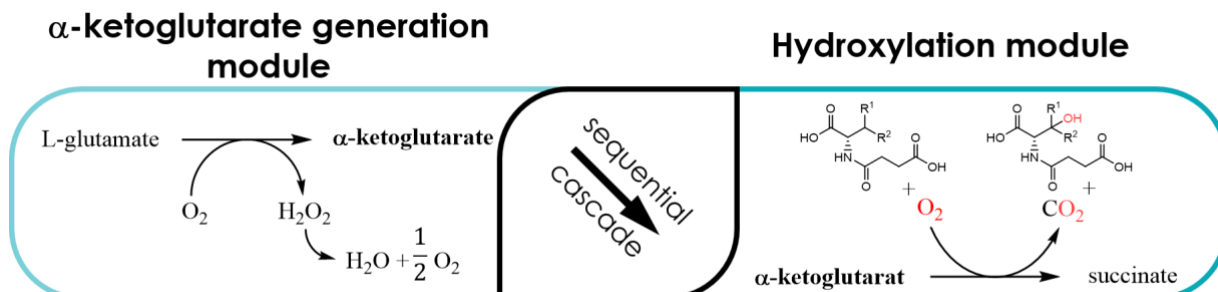
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# On the way towards cofactor supply via flow Biocatalysis

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## Abstract:

Enzymatic oxidation has a great potential for industrial application due to the ability to oxidize unactivated compounds stereo- and regio-specifically. However, oxidative biotransformations are still not frequently applied at industrial scale. Oxidative enzymes regularly show limited compatibility with chemical process conditions such as high substrate concentrations, solvent use and presence of strongly oxidative conditions. One of the highly interesting oxidative enzymes are α-ketoglutarate (αKG) dependent dioxygenases. Irreversible decarboxylation by oxidation of αKG leads to activation of the oxygen, which can hydroxylate various compounds.

As αKG is crucial for the catalytic cycle of these enzymes, the availability of this cofactor was investigated in this study in a model reaction. For the αKG production L-glutamate can be oxidized enzymatically by the oxygen dependent glutamate oxidase<sup>1</sup>. This reaction was coupled with an amino acid hydroxylase<sup>2</sup> for further studies of the realisation of this cascade process. Different strategies were studied regarding the enzyme formulation (cell free extract, purified or immobilized enzyme) as well as reactor configurations.

The most promising approach was identified as being a one pot sequential cascade using immobilized enzymes. This was tested in stirred tank batch reactors and will be transferred to a flow system for better recyclability of the involved enzymes. A scheme showing the modules of the cascade is shown in Figure 1.

**Figure 1:** Scheme of the cascade modules for the biotransformation. L-glutamate is oxidized to αKG followed by addition of the amino acid for the hydroxylation of the dioxygenase.

**Keywords:** dioxygenase, hydroxylase, flow chemistry, industrial biotechnology, cofactor supply, oxyfunctionalization, cascade, immobilization

**Session:** Bioprocess Engineering

## References:

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# Endophytic fungi as robust biocatalysts to oxyfunctionalize hydrocarbon terpenes

F.Cecati,<sup>1</sup> M.Kurina-Sanz,<sup>1\*</sup>

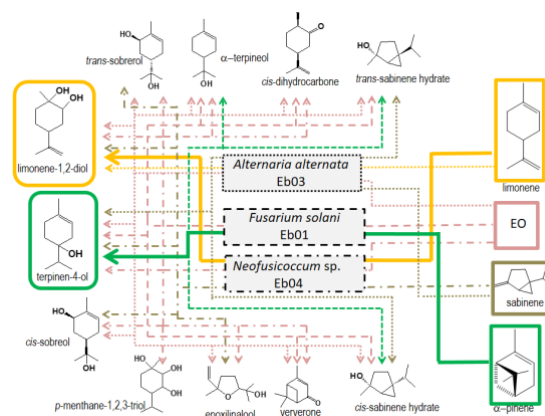
<sup>1</sup> INTEQUI-CONICET, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina.

## Abstract:

Biogenic volatile organic compounds (BVOCs) are industrially relevant biomaterials. Some of them, as limonene or pinene, are important platform chemicals. In the last decades, old and new technologies have been optimized for their extraction from agro-industrial wastes and abundant natural feedstocks. Synthetic biology provide other attractive alternatives to produce several mono- and sesquiterpenes by fermentation mediated by metabolically engineered microbes. More recently, elegant cell-free biochemistry platforms are emerging promising to significantly increase the titres of a few C<sub>10</sub>H<sub>16</sub> olefin hydrocarbons since overwhelm the cell toxicity problems.<sup>1</sup> The selective oxyfunctionalization of hydrocarbon BVOCs correlates with the increase of their qualities as flavours, fragrances and bioactivity as well as with the expansion of their uses as building blocks. Since redox reactions are involved requiring a high degree of specificity, whole cell biotransformations are good alternatives. However they have the disadvantage that these substrates are naturally antimicrobial. Our hypothesis is that endophytic microorganisms possess the ability to survive in the presence of BVOCs produced by the host plants of which they come from, because they have developed metabolic skills to biotransform antimicrobial components. In the present work we recovered endophytes from a South American endemic plant, *Eupatorium buniifolium*, that produce a monoterpene enriched essential oil (EO). Three fungal strains showed the ability to tolerate, and therefore biotransform, both the EO and four of its main pure components *R*-(+)-limonene,  $\alpha$ -(-)-pinene,  $\alpha$ -(+)-pinene and sabinene. The strains were characterized as *Fusarium solani* Eb01, *Alternaria alternata* Eb03 and *Neofusicoccum* sp. Eb04 by genomic and proteomic methods. Using these biocatalysts as resting cell, we accessed to three new complex volatile matrixes with different aroma notes from the original EO. Moreover, the bases of a couple of biotechnological processes to obtain

enantiomerically pure terpinen-4-ol and limonene-1,2-diol were set up using different sources of pinene and limonene as substrates, respectively. This work shows that certain endophytic microorganisms are suitable to enlarge the tool-box for the generation of valuable bioproducts from hydrocarbon BVOCs due to their robustness and biocatalytic skills.

**Keywords:** biotransformation, endophytic fungi, monoterpenes, biooxidation, BVOCs.



**Figure 1:** Biotransformation pathways from the EO and its main hydrocarbon monoterpenes. Solid lines denote the biooxidations involved in terpinen-4-ol and limonene-1,2-diol production bioprocesses.

## References:

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# The use of Laccase in a biotechnological approach for the degradation of aflatoxins

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<sup>1</sup>INRS-Institute Armand-Frappier, Laval, Qc, Canada

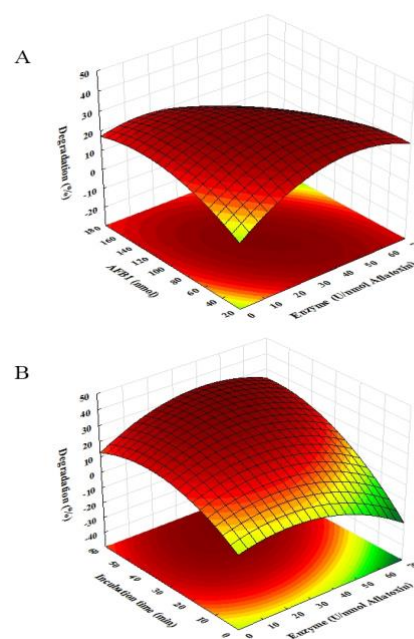
<sup>2</sup>McGill University, Ste-Anne de Bellevue, Qc, Canada

## Abstract:

Aflatoxins, which are highly toxic, mutagenic, teratogenic and carcinogenic compounds, are produced as secondary metabolites by fungi belonging to several *Aspergillus* species. These toxins have been widely studied because of their toxic, mutagenic, teratogenic and carcinogenic properties for human and animal health. The biocatalysis of laccase, from *Coriolus hirsutus*, using the major aflatoxin isoforms, AFB1, AFB2, AFG1 and AFG2, as substrates obtained from *Aspergillus parasiticus*, was investigated. The results showed a specific activity of 0.133, 0.124, 0.155 and 0.138  $\mu\text{mol}$  of degradation product per mg protein per min, for AFB1, AFB2, AFG1 and AFG2, respectively. The kinetic studies indicated that the  $K_m$  value was 0.646, 0.128, 0.237 and 0.284  $\mu\text{M}$  and a  $V_{\text{max}}$  value was 10.40, 9.68, 13.00 and 10.80 for AFB1, AFB2, AFG1 and AFG2, respectively. In addition, the presence of 0.4 mM of dithiothreitol and diethyldithiocarbamic acid strongly inhibited the laccase activity, whereas 10 mM of kojic acid and 0.01 mM of *p*-coumaric acid greatly promoted its activity. Moreover, the optimization of the laccase-catalyzed degradation using the aflatoxins as substrates with response surface methodology (RSM) and a five-level by three factors central composite design (CCD) was investigated. The results showed an enzymatic degradation rate of 38.2, 30.1, 76.4 and 100.0% for AFB1, AFB2, AFG1 and AFG2. Using HPLC, FTIR and LC/MS, the structural characterization of laccase-catalyzed end products and the residual aflatoxins was investigated. The results suggest that the mechanism of aflatoxins laccase-degradation could be the epoxydation, hydroxylation, *o*-demethylation, dehydrogenation, dehydration, reduction of the double bond and keto-reduction as well as the loss of ketone, oxygen, carbon and methyl molecules which could lead to the modification of either furfuran moiety, coumarin or the lactone ring as well as to the formation of nontoxic products. Overall, the experimental findings support the potential use of laccase in a biotechnological approach for the detoxification of food and animal feed.

**Keywords:** Aflatoxins, partial purified laccase, degradation, kinetic parameters, activators, inhibitors,

optimization, response surface methodology, central composite design, mechanism of degradation.



**Figure 1:** Response surface 3-D plot showing the effects of the independent variables on AFB1 degradation. (A) Effect of enzyme concentration (U/nM Aflatoxin) and AFB1 concentration (nM) while keeping incubation time ( $X_3$ ) constant and (B) Effect of enzyme concentration (U/nM Aflatoxin) and incubation time (min) while keeping substrate concentration ( $X_2$ ) constant.

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# **Biotech France 2018 - Session II: Environmental Biotechnology - Green Economy**



# Refactoring carbon metabolism of microbial systems for added value chemicals production from renewable carbon sources

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## Abstract:

The development of carbon efficient pathways for added value (bio)chemicals production is the essence of White Biotechnology. This biotechnological objective is at variance of that of microbial cells which have developed their metabolism essentially for growth or biomass production. It is therefore conceivable that the existing biochemical pathways which have been evolved over millions years could be refactored for dedicated biotechnological purposes. IN this respect, we have recently designed, constructed and expressed from scratch a synthetic pathway for the production of a methionine precursors from glucose [1]. In addition, frequently, natural metabolic networks do not have the stoichiometric capacity to produce a value-added compound at yields that correspond to the thermodynamic maximum. A good example of natural metabolic networks lacking stoichiometric efficiency is the bioproduction of glycolic acid (GA), a two carbon compound of considerable industrial interest notably in cosmetics and biodegradable polymers. We addressed this objective to approach this maximal conversion yield by employing the following strategies. Firstly, we reconsider a completely different route of C5 assimilation that by-passes the decarboxylation reaction in the pentose phosphate pathway and that relies on the carbon-conserving aldolytic cleavage of X1P or R1P to yield the C2 compound glycolaldehyde and the C3 DHAP compound. This metabolic scheme required the expression of human hexo(fructo)kinase (Khc-C) and human aldolase (Aldo-B). Then glycolaldehyde can be either reduced by endogenous aldehyde reductase to produce ethylene glycol or oxidized into glycolic acid. With this approach, we obtained yield of EG and GA close to maximal theoretical yield of 1 mol/ mol sugar [2, 3]. Interestingly, we found that the engineered strain expressing this synthetic pathway exhibited a remarkable rewiring of the metabolic networks that culminate with a dramatic reduced

metabolites and metabolic energy levels. In a second stage, we combined this synthetic pathway with the natural glyoxylate shunt that can be engineered to produce GA from DHAP. This combination led to an optimized production strain that produced ~30 % more GA from a xylose/glucose mixture (66 %/ 33 %) than when the natural pathway is working alone [4]. Finally, in a third stage, we designed a new cycling pathway that allows to reach in theory the maximal yield of glycolic acid production from either glucose (3 moles GA/mole) or pentose (2,5 mole/mole).

**Keywords:** synthetic biology, metabolic refactoring; thermodynamic, carbon conservation, glycolic acid, white biotechnology.

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# Biotechnology Innovation Observatory

Ronnie F. de Brito,<sup>1</sup> Milton Shintaku,<sup>1</sup> Diego J. Macedo,<sup>1</sup>

<sup>1</sup> Instituto Brasileiro de Informação em Ciência e Tecnologia, Brasília, Brazil

**Abstract:** Biotechnology is an interdisciplinary field, merging knowledge from different knowledge areas. Such diversity of sources and forms of representation gives researchers and practitioners in this field, and those in the field, challenges for their understanding under a broader vision. In order to promote development and innovation in the adoption of biotechnologies, it is proposed the structuring of an information system to identify, map and present different sources of information, opportunities for application, productive arrangements, challenges and barriers in biotechnology related areas. This information system is characterized as an observatory, which applies a methodology of data management aimed at the organization of information produced, derived or associated with research in biotechnology. Specifically, it is conceived according to two approaches: one methodological and one technological. The methodological approach consists of the definition of the partners and agents involved in the observatory, the definition of strategies for producing, sharing, analyzing and using data on biotechnologies. Among the methodological strategies are the use of controlled vocabularies, the use of a classification of the types of biotechnologies and the integration with dictionaries of research areas, such as CASRAI and CERIF and CNPq Lattes. The technological approach seeks to structure the information architecture of the observatory from the point of view of users, to elaborate the artifacts involved in the consolidation of information from data sources, and to propose formats for its presentation. Thus, the observatory should enhance the comprehensiveness and visibility of research results and technological developments, providing grounds for the elaboration of public policies to advance the application of biotechnologies in areas such as agriculture, medicine, the environment and others.

**Keywords:** biotechnology observatory, information system, knowledge management, information architecture, data integration, terminology.

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**Acknowledgment:** This project is sponsored by the Ministério de Ciência, Tecnologia, Inovações e Comunicações and implemented by the Instituto Brasileiro de Informação em Ciência e Tecnologia, with the support of the Fundação de Desenvolvimento da Pesquisa.



# Statistical optimization of chitin extraction from mushroom wastes by lactic acid fermentation in a stirred tank bioreactor

N. Patra<sup>1,2</sup> and N. Abu-Ghannam<sup>1</sup>

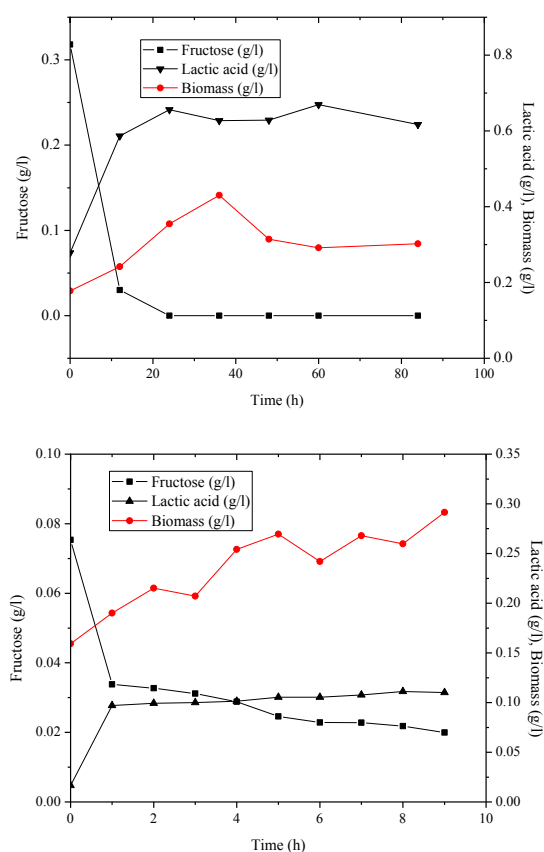
<sup>1</sup>School of Sciences and Health, Dublin Institute of Technology, Republic of Ireland.

<sup>2</sup>Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, India

## Abstract:

Chitin can be obtained using chemical degradation methods using crab and shrimp shell wastes. The process of extraction is wasteful and provides limited supply due to the seasonal availability. In the present study, mushroom wastes were subjected to fermentation-assisted extraction of value-added products such as chitin. The lactic acid fermentation process was optimized using statistical tools such as Plackett-Burman Design and Response Surface methodology and the optimal values of solid to liquid ratio was 60 g/l, inoculum size was 10% v/v and temperature was 30 °C. Batch fermentation of mushroom wastes was also performed using the optimized conditions in shake flasks and 7-L stirred tank bioreactor (STR). The experimental data obtained from the kinetic study was further utilized to determine the values of growth kinetic parameters. It was observed that at the end of 24 h the final *Lactobacillus paracasei* dry cell weight obtained in the stirred tank bioreactor was 0.43 g/l. Crude chitin harvested from the bioreactor was 0.15 g/g of mushroom wastes on dry cell weight basis and the residual mineral content was found to be 3.8 %. The growth kinetic data values determined from STR study will be utilized for further improvement of large-scale production of chitin using mushroom wastes.

**Keywords:** Chitin, *Lactobacillus paracasei* fermentation, Statistical design, Batch cultivation, Kinetic studies.



**Figure 1:** Figure illustrating growth kinetic studies of batch culture of *Lactobacillus paracasei* growing on mushroom wastes in a shake flask (Top) and 7-L stirred tank bioreactor (Bottom)

## References:

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# From BioSciences to BioProduction: The Pre-Industrial Demonstrator TWB

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## Abstract:

Toulouse White Biotechnology (TWB) is a public Joint Service Unit managed by INRA under the triple supervision of INRA, CNRS and INSA. Its mission is to help private companies to address the challenge of climate change. TWB is working hand in hand both with industry and top scientific level academics in view to develop sustainable processes based on the biotransformation of renewable resources. TWB has built a high performance interface to accelerate the transfer of innovative scientific results into efficient products and processes, as well as the offering of state of the art scientific answers to the strategic industrial issues.

TWB is ruled by a private/public consortium agreement which significantly simplifies and accelerates the signature of research contracts.

Every year TWB grants 3-4 blue-sky, fundamental projects to generate original IP which is available in priority to the industrial members of its consortium. These projects are examined under the scope of sustainability and ethics within the frame of a collaboration with the Higher School of Ethics of Sciences of Toulouse.

Since 2012, TWB has managed 105 R&D projects, among which 45 projects with private companies for a total amount of 21 M€. Several projects developed at the industrial and/or industrial pilot scale will be described.

TWB is presently hosting four start-up companies (EnobraQ, Pili, MicroPep, BFC) and has contributed to the rising of ca. €100m venture capital funds by such SMEs.

**Keywords:** industrial biotechnology, synthetic biology, biocatalysis, bioproduction, process scale-up, downstream processing.



Figure 1: Public/Private TWB's Consortium.

## References:

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# Environmental impact of bio-based products: considerations from a life-cycle perspective

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## Abstract:

Human influence on the climate system is clear, and recent anthropogenic emissions of greenhouse gases are the highest in history. Continued emission of greenhouse gases will cause further warming and long-lasting changes in all components of the climate system, increasing the likelihood of severe, pervasive and irreversible impacts for people and ecosystems [1]. Limiting climate change would require substantial and sustained reductions in greenhouse gas emissions which, together with adaptation, can limit climate change risks [1]. In addition, fossil fuel depletion has been identified as a future challenge for our society.

Biomass is an abundant renewable resource that can be used for the production of biofuels, biochemicals and bio-based materials, such as bio-polymers and bio-fibers. Bio-based products are promising sustainable alternatives for petroleum based materials. Firstly, they are made from renewable feedstock, providing the opportunity to create a balanced system of CO<sub>2</sub> sequestration and CO<sub>2</sub> emission with reduced net greenhouse gas emissions. Many studies have shown that bio-based chemicals and plastics can have significant environmental performance advantages over petrochemicals, especially with respect to greenhouse gas emissions [2]. Secondly, products made from biomass do not use petroleum as a feedstock, thus reduce the dependence on finite fossil resources. To build a bio-based production industry in which fuels, energy and materials, currently mostly generated from fossil resources, are incrementally replaced by equivalent or novel products from renewable resources, will require sustainably harnessing the vast biomass resource [2].

Life Cycle Assessment (LCA) is a recognized and a standardized methodology [3] to quantify the environmental impacts of a product or a service. LCA is used to quantify global warming potential, but also a wide range of other environmental impacts, for example acidification potential and eutrophication potential. LCA has the advantage of considering the entire life cycle of a product and is therefore a versatile technique

to avoid shifting environmental impacts from one life cycle stage to another stage. Since LCA can be used to assess several environmental consequences of a product or service, it can also support decision makers to avoid creating a new environmental issue while solving the current environmental issues.

In our research, LCA is used to compare the environmental impact of bio-based products with their fossil based counterparts, but also to gain insight in how the environmental performance of bio-based products can be improved while the products are still under development on laboratory or pilot scale. In addition, considering the full life cycle of a bio-based product can reveal how the environmental performance can be improved by optimizing the whole value chain. We will present LCA studies of bio-based products to illustrate how LCA can support the development of sustainable bio-based products.

**Keywords:** bio-based products, environmental impact, Life Cycle Assessment, greenhouse gas emissions, sustainable products

## References:

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# Engineering structural composites using bio-based materials

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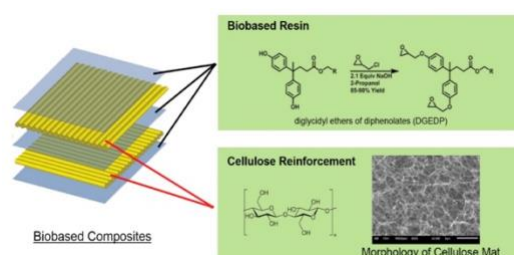
## Abstract:

High performance epoxy thermoset composites are ubiquitous all around us. Their light weight, high strength and great chemical resistance make them the material of choice in aerospace, automobile and wind turbine like applications. Due to the impending energy crisis and climate change caused by petroleum-derived carbon, there is an increasing demand for the plastic industry to replace petroleum based materials by readily renewable biobased feedstock. This implies replacing the matrix with its biobased counterpart as well as finding suitable high strength alternatives to glass and carbon fibers used in conventional structural composites. Bacterial Cellulose (BC) and Microfibrillated Cellulose (MFC), both derivatives of the most abundant renewable material known to man were chosen as our replacements to traditional reinforcements. Their excellent strength-to-weight ratio, low cost and synonymous refractive index to epoxy make them suitable materials of choice. Moreover, the morphological differences observed during preparation of the two mats provides a unique opportunity to study the effect this has on final thermo-mechanical and optical properties.

In parallel, a bio-based epoxy resin derived from diphenolic acid (DGEDP) was engineered to replace DGEBA (diglycidyl ether of bisphenol-A) for vacuum infusion molding. Curing kinetics namely, the viscosity rise and gel time were tuned to facilitate composite manufacturing. Finally, a complete bio-based composite made from DGEDP epoxy and cellulose fiber mats was prepared and compared with a traditional

glass fiber composite to illustrate its potential use in the future.

**Keywords:** Bacterial cellulose; Microfibrillated cellulose, biobased, green structural composites, processing, cure kinetics



**Figure 1:** Figure illustrating the biobased components of a green structural composite.

## Reference:

1. L. Yue, A. Maiorana, A. Patel, R. Gross, I. Manas-Zloczower, (2017) "A sustainable alternative to current epoxy resin matrices for vacuum infusion molding", *Composites Part A: Applied Science and Manufacturing*, 100, 269-274

# Production and use of microbial biomass helping sustainability in fish production chain

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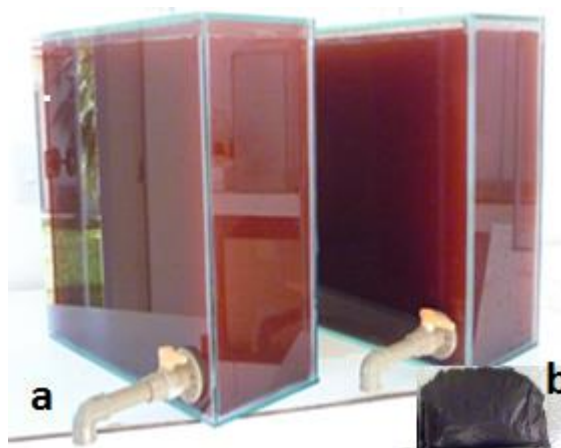
<sup>4</sup>Centro Universitário de Adamantina, Adamantina SP, Brasil

## Abstract:

This study represents an example of the classical concept of biotechnology as “the use of living organisms to make useful products and provide services”. Its purpose was to investigate the ability of phototrophic bacterium *Rubrivivax gelatinosus* to cause the depollution of an industrial byproduct and work as an ingredient for animal feed as a way of making the productive process more sustainable. First, the bacterium was grown in fish industry effluent (inoculum at 1% v/v, 30 ± 5 °C, 2000 ± 500 lux, 7 days) and recovered as a biomass (microfiltration + centrifugation + lyophilization) (Figure 1), resulting in a decrease of ca. 80% in the Chemical Oxygen Demand of the pollutant byproduct and so putting it within the required limits for wastewaters discard. Next, the acute toxicity of the biomass was investigated in rats using the bacterial biomass at 300, 1,000 or 2,000 mg/kg body weight via gavage at single dosing. The physiological and clinical (hippocratic screening) signals of the animals were recorded during 15 days. As no deaths occurred and no signals of adverse effects were observed at any dose of the biomass, it was characterized as a non toxic product. The physicochemical investigation indicated nutritional and technological properties in the bacterial biomass due to its composition: 4% moisture, 5% ash, 46% protein, 17% lipids and 0.3% red oxycarotenoids. Further, the biomass was used as an ingredient to feed cultured tilapias (175, 350, 700 or 1,400 mg/kg, water recirculation system, 80 days) providing increases on the color (a\*) and the protein content (micro Kjehdahl method) of the fillets, besides a delay on the meat rancidity (Thiobarbituric Acid Reactive Substances) (Table 1). The use of the ingredient increased the carotenoids contents of the fillets, arising a possibility to the use of the fillets as a functional food, since the carotenoids are related to the prevention of several diseases. It was

demonstrated the biotechnological application of *R. gelatinosus* biomass, as a contribution to the sustainability in fish raising and processing and so providing benefits to environment, industry and consumers.

**Keywords:** *Rubrivivax gelatinosus*, tilapia, industry wastewaters, oxycarotenoids, proteins.



**Figure 1** - Cultivation of *R. gelatinosus* in fish processing wastewater (a) and the final aspect of the biomass (b).

**Table 1** - Features of the fillets of tilapias fed diets with *R. gelatinosus* biomass

Treatment	redness	protein (%)	carotenoid (mg/kg)	rancidity 80 d (mg MA/kg)
control	1.32 ± 0.16 <sup>c</sup>	18.02 ± 0.46 <sup>b</sup>	3.34 ± 0.41 <sup>b</sup>	0.807 ± 0.028 <sup>a</sup>
T1	1.88 ± 0.14 <sup>b</sup>	19.49 ± 0.43 <sup>a</sup>	5.30 ± 0.21 <sup>a</sup>	0.604 ± 0.034 <sup>b</sup>
T2	2.26 ± 0.19 <sup>a</sup>	19.43 ± 0.42 <sup>a</sup>	5.29 ± 0.22 <sup>a</sup>	0.468 ± 0.044 <sup>c</sup>
T3	2.59 ± 0.18 <sup>a</sup>	19.53 ± 0.58 <sup>a</sup>	5.69 ± 0.90 <sup>a</sup>	0.453 ± 0.036 <sup>c</sup>
T4	2.55 ± 0.10 <sup>a</sup>	19.69 ± 0.53 <sup>a</sup>	5.79 ± 0.54 <sup>a</sup>	0.459 ± 0.039 <sup>c</sup>
p value	< 0.0001	0.0013	< 0.0001	< 0.0001

T1 – 175 mg biomass/kg diet; T2 - 350 mg biomass/kg diet; T3 - 700 mg biomass/kg diet; T4 – 1,400 mg biomass/ kg diet. MA – malonaldehyde. Tukey's test (p<0.05).



# Pesticides in the Environment and Mitigation Technologies

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<sup>1</sup>University of La Frontera, Department of Chemical Engineering, Temuco, Chile

<sup>2</sup>University of La Frontera, CIBAMA-BIOREN, Temuco, Chile

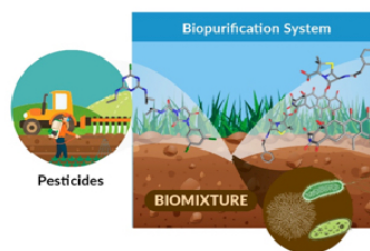
<sup>3</sup>University of La Frontera, Department of Chemical Sciences and Natural Resources, Temuco, Chile.

## Abstract:

The rapid increase in demand of pesticides to sustain and improve quality of life worldwide has resulted in the contamination of air, water, and soils, posing a potential threat to the environment and a health concern in many communities, making their removal and detoxification a more urgent undertaking. The time that pesticides remain in the environment depends on how strongly they are bound by soil components, how readily the soil microflora degrades them, as well as by chemical characteristics of the pesticides. Several technologies available for the treatment of pesticide-contaminated sites have been reported (Karanasios *et al.* 2012). Castillo *et al.* (2008) present the state of the art of biobeds system, a biopurification system widely used in Sweden and others countries in Europe to avoid or minimize point-source contamination during on-farm manipulation of pesticides. This technology is based on the adsorption and degradation potential of an organic biomixture composed of topsoil, peat, and straw and covered with a grass layer (Castillo *et al.*, 2008). Straw stimulates growth of ligninolytic microorganisms and the production of extracellular ligninolytic enzymes. Peat contributes to sorption capacity, moisture control, and abiotic degradation of pesticides and decreases pH of the biomixture, which is favorable for fungi and their pesticide-degrading enzymes (Castillo *et al.*, 2008). Soil enhances the sorption capacity in the biobed and provides an adequate mixture of microorganisms. The grass layer that covers a biobed is relevant to enhance of pesticide degradation due to interactions between microorganisms and root exudates. The exudates increase microbial activity and community numbers in the rhizosphere and can influence the dissipation of chemical by different pathways (Diez *et al.*, 2017).

We evaluated the influence of different parameters (components of the biomixture, pesticides characteristics, operational conditions of the biopurification system, effect of the

rhizosphere, effect of bioaugmentation, etc.) on the degradation of a mixture of pesticides



**Figure 1:** Graphical abstract illustrating the biopurification system.

**Keywords:** biopurification system, pesticides, rhizosphere

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3. Diez, M.C., Leiva, B., Gallardo, F. (2017). Novel insights in biopurification system for pesticides mixture dissipation in repeated applications. *Environmental Science and Pollution Research*. DOI 10.1007/s11356-017-0130-z.

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**Biotech France 2018 - Session III:  
Pharmaceutical  
/ Medical Biotechnology**



# Implementation of bacterial expression systems for the detection of L-asparaginase activity in droplet-based microfluidics

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## Abstract:

L-Asparaginase is an enzyme that primarily catalyzes the conversion of L-asparagine to L-aspartic acid and ammonia. Currently, L-asparaginase originating from *E. coli* and *Erwinia chrysanthemi* are used as drugs in the treatment of acute childhood lymphoblastic leukemia (ALL)<sup>1</sup>. However, the use of L-ASNase in clinical treatment often generates side effects resulting to interrupting treatment<sup>2</sup>. The human genome codes for at least three enzymes able of hydrolyzing the amino acid L-asparagine. They do not present sufficient catalytic activity to be administered in therapeutic treatment<sup>3</sup>. One strategy to circumvent side effects is to engineer the human asparaginase using directed evolution methods.

Directed evolution experiments is limited by the screening technique employed. The emergence of high-throughput screening (HTS) techniques such as droplet-based microfluidic is a revolution to improve large libraries analysis. Droplet-based microfluidic technology consists in controlling monodisperse water in oil droplets at the single droplet level<sup>4</sup>. One of the main principal advantage of this technology is the capability to maintain cell viability during and after the process of screening and sorting. In this way, sorted cells presenting interesting properties can be directly culture for overexpression and characterization of the optimized enzyme.

In this study, we developed and compared three expression systems of the EcASNase2 in *E. coli*: cytoplasmic, periplasmic or Anchored Periplasmic Expression (APEX). Our aim is to have an sufficient amount of recombinant protein produced per single cell with a correct accessibility for the substrate in order to optimize the detection time of enzymatic activities in view of screening of enzymes with low catalytic activity. We used a three-step coupled enzyme assay using the natural substrate of the enzyme, L-Asn, and resulting to the oxidation of the Amplex Ultra Red into fluorescent resorufin<sup>5</sup>.

The fluorescence of each droplet was monitored at different measurement point using a 532 nm laser to reconstruct single cell kinetics. Our results show that periplasmic expression is the best choice for directed evolution experiments as they guarantee both a high level of expression, an accessibility of the enzyme and the cell viability during the screening process.

**Keywords:** L-asparaginase, Acute Lymphoblastic Leukemia, Expression systems, Droplet-based microfluidic, Directed evolution.

## References:

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2. N. Hijiya & I. M. van der Sluis, 2016. Asparaginase-associated toxicity in children with acute lymphoblastic leukemia. *Leukemia & Lymphoma*, 57(4), 748–757.
3. C. S. Karamitros & M. Konrad, 2016. Fluorescence-Activated Cell Sorting of Human l-asparaginase Mutant Libraries for Detecting Enzyme Variants with Enhanced Activity. *ACS Chemical Biology*, 11(9), 2596–2607.
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# Targeting Cancer at the Nuclear Pore

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## Abstract:

Trafficking of biological materials across nuclear membrane is an evolutionarily conserved mechanism that maintains normal eukaryotic cell homeostasis. Smaller entities can enter and exit the nuclear pore through diffusion. The movement of most of the proteins however, requires active transport that is mediated by specialized carriers called Karyopherins that maintain proper compartmentalization of micro- and macromolecules. Such energy dependent trafficking occurs through the nuclear pore complex (NPC) that is embedded in the nuclear membrane. This is functionally critical for tumor suppressor proteins (TSPs) and transcription factors (TFs) that require nuclear retention and sequence specific DNA alignment to modulate their target gene expression or conduct genome surveillance activity. Indeed, cancer cells have evolved methods to disturb the nuclear traffic by abnormal expression of the nuclear exporters particularly exportin 1 (XPO1) that leads to a cascade of de-regulations favoring uncontrolled growth and loss of surveillance within the cells. Recently, specific inhibitors of nuclear export (SINE) have been developed as a broad form of therapy targeting global re-alignment of multiple TSPs in the correct cellular compartment through inhibition of XPO1 to rein in cancer. SINEs are under extensive Phase I and Phase II clinical evaluation for various tumor indications. Here the proof of concept is presented using the case of pancreatic ductal adenocarcinoma (PDAC).

XPO1 was found to be over-expressed in PDAC but not normal pancreas tissue. SINE compounds but not inactive analog induced PDAC cell death and synergized with gemcitabine (GEM) and nab-paclitaxel leading to enhanced PDAC growth inhibition, apoptosis, and spheroid disintegration of PDA derived cancer stem

cells (CSCs) (CI<1). The observed synergy was due in part to enhanced nuclear localization of TSPs. Crispr Cas9 mutant cells lacking SINE binding cys528 were not responsive to treatment confirming specific role of XPO1 in the reaction. Selinexor enhanced the anti-tumor efficacy of gemcitabine or nab-paclitaxel in patient derived xenograft. Evaluable responses were observed in patients on this trial. More specifically, one patient with stage IV metastatic PDAC and high CA19-9 levels at diagnosis demonstrated an outstanding objective response. His symptoms significantly improved with a marked reduction of CA19-9. He was in remission for 16 months and remains alive 21 months after starting therapy. Published median progression free and overall survival times in patients on gemcitabine-nab-paclitaxel are below 6 and 9 months, respectively. In conclusion our in vitro, in vivo and Phase I clinical studies strongly support the development of nuclear export inhibitors as a viable therapy for PDAC and possibly other malignancies.

**Keywords:** Nuclear Protein Export, Exportin 1, XPO1, SINE, Pancreatic Ductal Adenocarcinoma.

**Data sharing:** Patient CT scans and CA19-9 parameters will be shared at the time of presentation.

# Reliability of 2D Kinematic Analysis of Sagittal and Frontal Plane Motion During Running in Individuals with Patellofemoral Pain

L. Cedin,<sup>1\*</sup> T.C. Rosa,<sup>1</sup> B.O. Peixoto,<sup>1</sup> D.H. Kamonseki,<sup>2</sup>

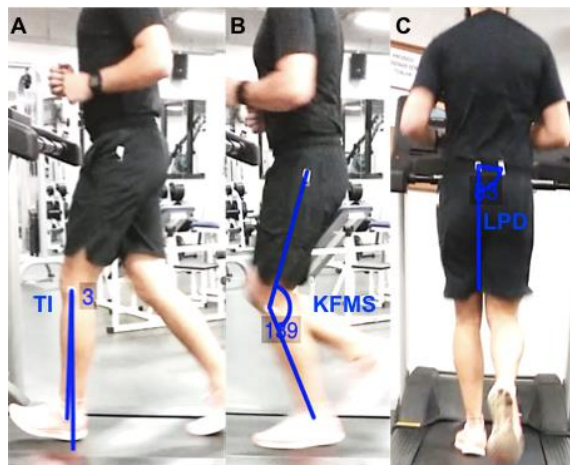
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<sup>2</sup> Universidade Federal de Sao Carlos, UFSCar, Department of Physical Therapy, Sao Paulo, Brazil

## Abstract:

Running has gained popularity worldwide however there has been a concomitant increase in injuries rates, specially on patellofemoral pain. Two dimensional video analysis has been proved to be reliable on healthy individuals, however, motion analysis of running-related injuries are needed in order to prevent injuries and elaborate rehabilitation programs. Therefore, the aim of the present study was to assess intra and inter-rater reliability of 2D video analysis for runners with patellofemoral pain. Running gait analysis videos were recorded from nine male and female recreational runners ( $36,2 \pm 9,6$  years old) by a high speed camera and rated on 2 different occasions by 2 experient evaluators. Volunteers completed a running protocol at a self-selected speed. Frame images were obtained at the same time – initial contact and midstance - and evaluated on Übersense Coach® app for each painful knee. Variables of interest included: lateral pelvic drop (LPD), tibial inclination (TI), knee flexion angle at initial contact (KFIC) and at midstance (KFMD) and trunk flexion (TF). Intra-Class Correlation Coefficients (ICC) were used to assess intra and inter-rater reliability of the users of the 2D software. The 2D testing method demonstrated excellent intra-rater reliability for LPD (ICC: 0.823), TI (ICC: 0.982), KFIC (ICC: 0.974), KFMS (ICC: 0.909) and TF (ICC: 0.942). TI (ICC: 0.930), KFIC (ICC: 0.871) and KFMS (ICC: 0.884) were highly reproducible between raters, however LPD (ICC: 0.407) and TF (ICC: 0.386) demonstrated poor inter-rater reliability. These findings suggest that 2D video analysis of frontal and sagittal planes in runners should be encouraged among clinicians, specially on those with patellofemoral pain. However, trunk flexion and lateral pelvic drop must be analysed with caution between raters.

**Keywords:** running, patellofemoral pain, lower extremity, motion analysis, video analysis, 2D video analysis.



**Figure 1:** Sagittal and frontal plane views of the whole body exhibiting variables analysed on patellofemoral pain knees. Figure shows (a) 3° of tibial inclination angle (TI), (b) 41° of knee flexion angle at midstance (KFMD) and 7° of lateral pelvic drop (LPD).

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# Genetic transformation of *Physcomitrella patens* to overexpress Pp-miR536

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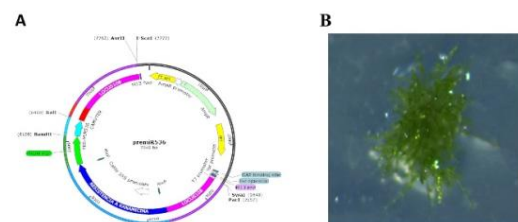
<sup>2</sup>Consejo Nacional de Ciencia y Tecnología - Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional (CIBA-IPN), México

## Abstract

Terrestrial plants that currently inhabit earth are classified as "non-vascular" and "vascular". Non-vascular plants, also known as bryophytes, are particularly interesting because of their phylogenetic relationship with the first plants that colonized earth around 500 MYA. Unlike vascular plants, bryophytes possess tolerance to several types of abiotic stress, including drought. Therefore, the study and the subsequent understanding of responses caused by drought in bryophytes are crucial for developing biotechnological tools toward the generation of crop plants highly tolerant to drought. Within the battery of mechanisms to counteract drought-derived effects, modulation of gene expression is crucial for restoring homeostasis. Particularly, microRNAs (miRNAs), which belong to a class of small non-coding RNAs, regulate gene expression through sequence complementarity. The hundreds of genes regulated by miRNAs are involved in several biological processes such as plant growth and development, but also in stress responses. For instance, the miR536 of *Physcomitrella patens* has recently been found to increase its expression during stages of recovery after drought treatment, suggesting a putative role in this process. Moreover, targets of miR536 are involved in Abscisic acid signaling, a well-known hormone involved in stress responses. Thus, the goal of this work is the study of the Pp-miR536 through increasing its expression by replacing the wild-type promoter with a strong and inducible promoter. The molecular and phenotypic

characterization of transformant lines overexpressing Pp-miR536 will decipher its function in adaptive responses during low water availability conditions.

**Keywords:** Plants, bryophytes, moss, *Physcomitrella*, transformation, microRNAs, stress, drought, overexpression.



**Figure 1. Genetic transformation of *P. patens*.** A) Schematic representation of the transforming vector pBNR-108-HSP-miR536. B) A regenerating moss colony over expressing Pp-miR536.

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## Acknowledgements

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# Posters Session

# Bioinformatic identification method of evolutionary ranges for functional small peptides on genomes

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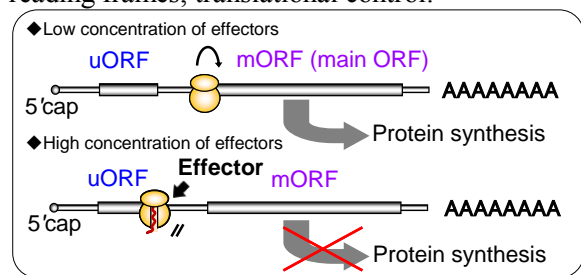
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## Abstract:

Upstream open reading frames (uORF) are small ORFs located in the 5' untranslated regions (5'-UTRs) of many eukaryotic mRNAs. Some uORFs encode functional peptides with evolutionarily conserved amino acid sequences. Comparative genomic approaches have been used in genomewide searches for uORFs encoding bioactive peptides, and by comparing uORF sequences between a few selected species or among a small group of species, conserved peptide uORFs (CPuORFs) have been identified in plants, mammals and insects. Regulatory regions within uORF-encoded peptides that are involved in translational control are typically 10–20 amino acids long. In conventional comparative genomic approaches, uORF sequences were compared between a few selected species, and, therefore, the detection of homology depends on the selection of species for comparison. To maximize the chances of identifying CPuORFs, we previously proposed the BAIUCAS method (BLAST-based algorithm), in which homology searches of uORF amino acid sequences are performed between a certain species and any other species whose transcript sequence databases are available. Here, we developed an improved version of BAIUCAS, designated as ESUCA, which can automatically determine taxonomic range of uORF sequence conservation. This newly added function enables us to efficiently select CPuORFs that are conserved in a wide taxonomic range and are likely to encode functional peptides. We applied ESUCA to five plant genomes and identified 97 novel groups of CPuORFs. We examined the sequence-dependent effects of several CPuORFs identified by ESUCA on mORF translation using transient expression assay, and three of seven CPuORFs conserved in a wide range of eudicots showed sequence-dependent inhibitory effects. Thus, ESUCA is a powerful method to comprehensively identify CPuORFs, and among

them, to select CPuORFs that are likely to encode functional peptides.

**Keywords:** bioinformatics, upstream open reading frames, translational control.



**Figure 1:** Translational control by uORF

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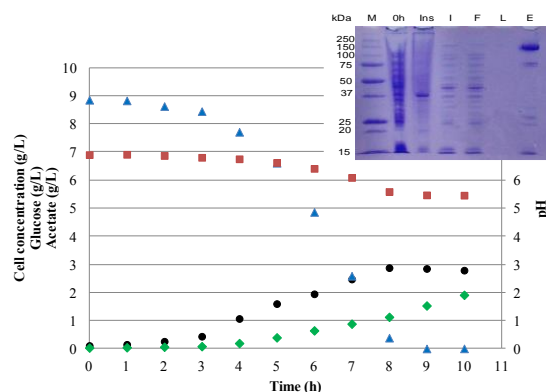
# Enhancing Cas9 nuclease expression in *Escherichia coli* using defined media

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## Abstract:

The CRISPR-Cas9 system has shown to be a promising new tool for manipulating DNA sequences and for genome editing. This system consists of a complex composed by an endonuclease Cas9 and a guide RNA (gRNA) that target the specific DNA sequence, enabling DNA cleavage and modification. The successful delivery of Cas9 protein and gRNA into cells for genome editing opens doors for the use of Cas9 protein as a therapeutic molecule. Studies evaluating the performance and viability of the scalable production of this recombinant protein as a biopharmaceutical have not been reported in the literature. Therefore, the aim of the present work was to study the culture conditions for the production of Cas9 nuclease in *E. coli*. We evaluated the cell growth and the expression of Cas9 nuclease in shake flask culture using a defined high cell density medium containing glucose. The kinetic parameters maximum specific growth rate ( $\mu_{\max}=0.53\text{h}^{-1}$ ) and substrate to cell yield factor ( $Y_{x/s}=0.30$ ) were determined at 37°C. The induction temperature and the post-induction time for the recombinant protein expression were also evaluated in order to optimize the Cas9 production. Our study demonstrates that, by using the optimized culture conditions, significant improvements in the expression of Cas9 nuclease in recombinant *E. coli* can be achieved.



**Figure 1:** Growth kinetic of *E. coli* in shake-flask culture at 37°C and defined media. SDS-PAGE of chromatographic steps to evaluate the Cas9 nuclease expression.

**Keywords:** Cas9 nuclease, recombinant protein expression, *Escherichia coli*, defined media, bioprocess.



# Novel Bioreactor System for Continuous IgG Production

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## Abstract:

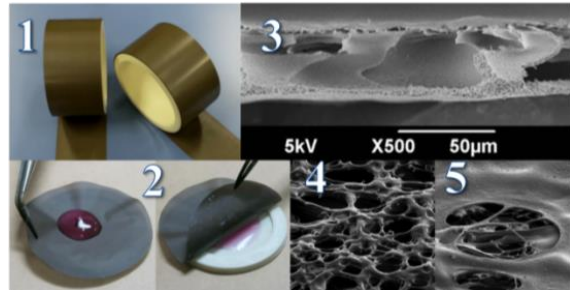
**Background:** A study of membrane cell culture utilizing materials comprised of porous polyimide (PPI). This membrane demonstrates capability for long term, stable mammalian cell culture. The developed cell culture system is capable of reproducible protein expression (IgG). The experiments summarized here outline the findings of repeated IgG expression in CHO DP-12 cells grown in modules containing the PPI membrane.

**Methods:** The bioreactor relies on the PPI membrane encased in a module. A module consists of a defined length of PPI membrane folded and encased into a polymeric shell of defined size. A module system consists of multiple modules placed into a given bioreactor. For the purposes of these experiments, the prepared modules are placed into a wave bag bioreactor system.

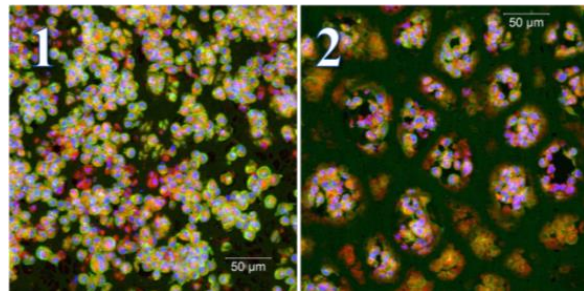
The modules with PPI membrane are placed into a 1-L wave bag system with media and inoculated with CHO DP-12 cells producing anti- hIL-8 antibody. The medium is harvested and fresh medium suitable for the attachment cell culture system is replenished continuously at a constant rate over a period of 5 months. Continuously produced IgG has been monitored by Cedex Bio System. The Quality of IgG was evaluated on High Performance Liquid Chromatography and SDS-PAGE gels.

**Results:** IgG production was found to be constantly expressed over the bioreactor operation period. The content and purity of the IgG before and after purification was assessed and found to be consistent over the 5 month period. A floating cell culture with the same CHO DP-12 cells was performed using shaker flask as the control experiment. The purity of the IgG was found to be consistent with that of the floating cell culture control case, the amount of IgG expressed in the module case was about 5 times or more, greater than the control.

**Keywords:** continuous protein production, long term stable cell culture, porous polyimide membrane, bioreactor for effective antibody production and stable quality



**Figure 1:** About our porous polyimide membrane 1, Appearance of the PPI 2, Water permeable profile of PPI membrane 3, SEM image of membrane cross section; This image presents the fact that this flexible thin membrane poses three dimensional macrovoid structure. 4 and 5, Upper and bottom face of the PPI membrane



**Figure 2:** CHO DP12 Cells grows in the PPI 1, CHO DP12 cells on the upper mesh face 2, CHO DP12 cells on the bottom big hole face

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# Isolation and selection of microorganisms capable of tolerating different pesticides for the construction of a microbial consortium

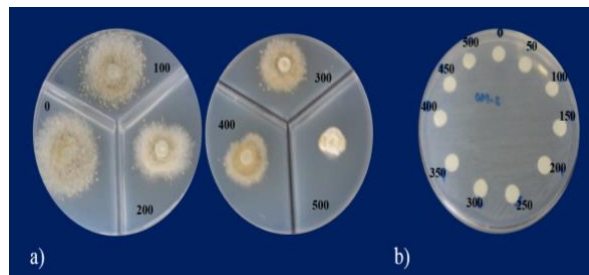
Rosa Arenas Saldaña<sup>1</sup>, Ángel E. Absalón<sup>1</sup> and Diana V. Cortés-Espinosa<sup>1\*</sup>

<sup>1</sup>Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada.  
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## Abstract

The need to produce enough food to supply a growing world population has led to an extensive use of pesticides in agriculture. Regardless of their benefits, pesticides are highly toxic substances that, given their mobility and persistence, enter into the food chain and create a significant impact on ecosystems, as well as in human health. Numerous decontamination techniques to eliminate pesticides from the environment have been investigated. Several works have used native organisms of the soil, exploiting their metabolic and enzymatic mechanisms, to treat these contaminants and promote their complete degradation into innocuous agents. Thus, the aim of this work was to isolate, identify, and select pesticide-tolerant microorganisms toward the goal of constructing a microbial consortium with a high pesticide degradation capacity. Bacterial (62) and fungal (25) strains were isolated from soils highly contaminated with pesticides. Such strains were subjected to different concentrations (0 to 500 mg l<sup>-1</sup>) of a mixture of pesticides (endosulfan, methyl paration, carbofuran and paraquat) (Fig. 1). Key findings showing the tolerance of our bacterial and fungal isolates are presented in this work. To our knowledge, this is the first study in which bacterial and fungal tolerant to extreme concentrations of different pesticides is evaluated, thereby opening the possibility to construct a highly tolerant and degrading microbial consortium.

**Keywords:** Pesticides, Soil Pollution, Microbial tolerance, Bioremediation, Degrading Consortium, Microbial diversity.



**Figure 1.** Pesticide-tolerance of fungal and bacterial isolates. **a)** Radial growth of isolated fungal strains in Petri dishes with different concentrations of a pesticide mixture (0-500 mg l<sup>-1</sup>). **b)** The radius of the inhibition halos of bacterial isolates in the presence of discs impregnated with different concentrations of a mixture of pesticides (0-500 mg l<sup>-1</sup>).

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## Acknowledgements

We appreciate the support of SIP-IPN, COFAA-IPN, CONACYT and the Instituto Politécnico Nacional for funding this work

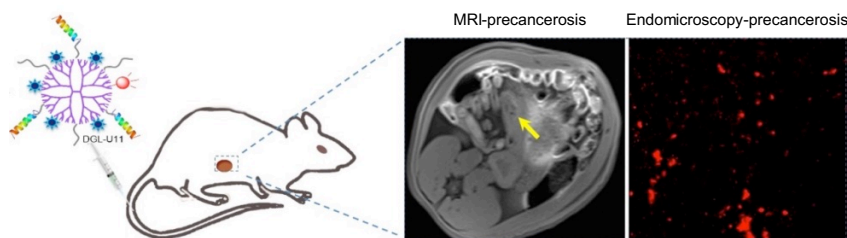
# Dendron-Grafted Polylysine-Based Dual-Modal Nanoprobe for Ultra-Early Diagnosis of Pancreatic Precancerosis via Targeting a Urokinase-Type Plasminogen Activator Receptor

Hui Li, Kai Yang, and Yingsheng Cheng\*

## Abstract:

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer death. Early detection of precancerous pancreatic intraepithelial neoplasia (PanIN) tissues is an urgent challenging to improve the PDAC prognosis. Here, we report a urokinase-type plasminogen activator receptor (uPAR)-targeted magnetic resonance (MR)/ near-infrared fluorescence (NIRF) dual-modal nanoprobe DGL-U11 for ultra-early detection of pancreatic precancerosis. Because of its good biocompatibility and biodegradability, globular architecture and well-defined reactive groups, the dendron-grafted polylysine (DGL) is chosen as the platform to load with a pancreatic tumor-targeting peptide U11, a magnetic resonance contrast agent  $Gd^{3+}$ -DTPA, and a NIR fluorescent cyanine dye Cy5.5. The nanoprobe DGL-U11 has several preferable characteristics, such as active peptide targeting to activator receptor, good biocompatibility, dual-modal imaging diagnosis, and well controlled diameter in a range of 15-25 nm. Upon incorporation of the active U11 peptide target to the over-expressed activator receptor uPAR, the targeted nanoprobe DGL-U11 can increase to the earlier PanIN-II stage through *in vivo* NIRF imaging. Labeled with both MR and NIRF bioimaging reporters, the uPAR-targeted dual-modal nanoprobe is very effective in the targeted imaging of precancerous PanINs and PDAC lesions with high sensitivity and spatial resolution, providing a promising platform to the ultra-early detection of PDAC.

**Keywords:** dendron-loaded nanoprobe, NIR fluorescence bioimaging, magnetic resonance imaging, pancreatic cancer, activator receptor.



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# Niemann-Pick Type C2 Protein Inhibits PDGF-BB-Induced Hepatic Stellate Cells Proliferation

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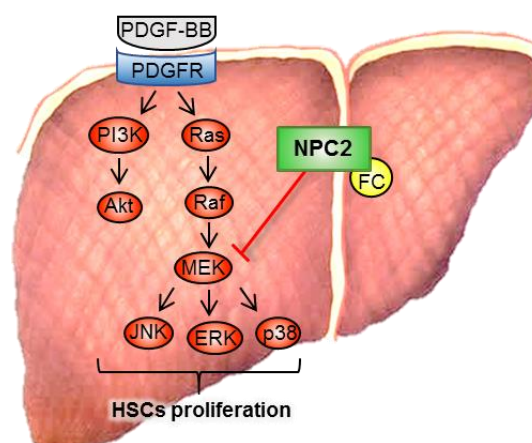
<sup>2</sup>Department of Biotechnology and Laboratory Science in Medicine, National Yang-Ming University, Taipei, Taiwan.

## Abstract:

**Background.** Liver fibrosis is the first step toward the progression to cirrhosis, portal hypertension and hepatocellular carcinoma. Hepatic stellate cells (HSCs) are the major profibrogenic cells that promote the pathogenesis of liver fibrosis. Platelet-derived growth factor BB (PDGF-BB) signaling is required for HSCs proliferation under pathological conditions. Furthermore, the toxin factor of microbes in the intestinal tract, which called lipopolysaccharide (LPS), will enter the liver and activate HSCs through NF- $\kappa$ B signaling pathway. Niemann-Pick type C2 (NPC2) plays an important role in the regulation of intracellular free cholesterol homeostasis via direct binding with free cholesterol. However, the roles of NPC2 in HSCs proliferation and inflammation have not been explored in detail. **Methods.** We used 293T cell to generate lentiviruses carrying NPC2 knock down and NPC2 overexpression genes. The HSC-T6 cells and LX2 cells were infected with the lentiviruses and selected long-term transfected NPC2 knock-down and overexpression cells using puromycin containing medium. The efficiency was confirmed by Q-PCR and Western Blotting. Furthermore, we used PDGF-BB and LPS treatments to compare the cell proliferation and inflammation, respectively. The molecular mechanisms focused on MAPK and NF- $\kappa$ B signaling pathways between (1) HSC-T6 shlacZ control and HSC-T6 shNPC2 cells; (2) LX2 eGFP control and LX2 NPC2 cells were analyzed. **Results.** In this study, we showed that knockdown of NPC2 in HSC-T6 cells resulted in marked increases PDGF-BB-induced cell proliferation through enhance ERK, p38 and JNK phosphorylation. In contrast, NPC2 overexpression decreased PDGF-BB-induced HSCs proliferation through inhibit ERK, p38 and JNK phosphorylation. However, the expression of NPC2 did not alter LPS-induced inflammatory signals and Nf- $\kappa$ B

phosphorylation in HSCs. In addition, U18666A-induced free cholesterol accumulation in LX2 cells increase cell proliferation and ERK phosphorylation, but not affect the activation of JNK and p38 (Figure 1). **Conclusion.** Our study demonstrates that NPC2 plays an important role in HSCs proliferation via regulates free cholesterol homeostasis.

**Keywords:** NPC2, PDGF-BB, HSCs



**Figure 1:** Proposed mechanisms by NPC2 inhibits HSCs proliferation in the liver.

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# Selenate Reduction to Elemental Selenium by *Shigella sonnei* strain SE6-1 and Its Potential Mechanism Elucidated by Genome Sequence

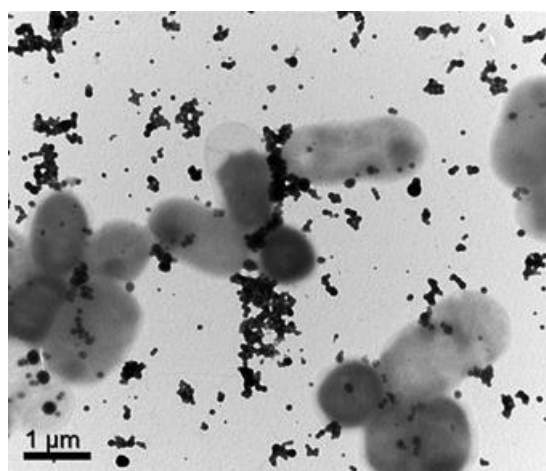
A. Cho,<sup>1</sup> H. Lee<sup>1</sup> and J-H. Lee<sup>1\*</sup>

<sup>1</sup> Department of Bioenvironmental Chemistry, Chonbuk National University, Jeonju, Republic of Korea

## Abstract:

Although selenium is an essential trace element, it is a potential toxic element to natural ecosystems above the essential amounts and/or concentrations due to its bioaccumulation potential. Selenium is often found naturally in the earth's crust, and emitted to atmospheric and aquatic environments, caused by various industrial activities such as mining-related operations. Among the technologies developed for the treatment of wastewaters containing selenium pollutant, biological selenium reduction has been suggested as one of the potential technologies for removing selenium from wastewaters since biological processes could be beneficial by being economical and environmental-friendly rather than many diverse physico-chemical processes to treat dilute and variable selenium-containing wastewaters. We have isolated selenate ( $\text{SeO}_4^{2-}$ )-reducing bacteria from a sewage drain sediment located in industrial complex area, city of Jeonju, South Korea. Among the isolates, we have selected the strain SE6-1 for further analyses on selenate reduction by screening and comparing the reduction activities of many isolates. Phylogenetic analysis on the strain using 16S rRNA gene revealed that the strain was closely related to *Shigella sonnei*. In anoxic condition, the strain SE6-1 reduced Se(VI) (aqueous) to Se(0) (elemental selenium) during anaerobic growth in nutrient-rich complex medium (Figure 1). It was not likely that the strain utilized selenate as the sole terminal electron acceptor for anaerobic respiration. Thus, we have analyzed genome sequence of the strain SE6-1 to propose the potential mechanism of the selenate reduction and found two genes coding putative selenate reductase. Biological reduction of selenium anions such as selenite and selenate may suggest a clue for biotechnological advances in treatment of selenium contaminated wastewaters, as suggested in this study.

**Keywords:** selenium removal, microbial selenium reduction, bioremediation of selenium contaminated wastewater, genome sequencing.



**Figure 1:** Transmission electron microscopic image of elemental selenium particles precipitated with the bacterial cells of strain SE6-1.

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# Improved shoot regeneration, salinity tolerance and reduced fungal susceptibility in transgenic tobacco constitutively expressing PR-10a gene

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## Abstract

Abiotic stresses such as drought and salinity exerts adverse effects on plant growth and development. To combat these stresses plants are having unique mechanism and it is largely regulated by plant hormones, which in turn, orchestrate the different biochemical and molecular pathways to manoeuvre stress tolerance. The PR-10 protein family is reported to be involved in defence regulation, stress response and plant growth and development. The JcPR-10a overexpression resulted in increased number of shoot buds in tobacco (*Nicotiana tabacum*), which could be due to high cytokinin to auxin ratio in the transgenics. The docking analysis shows the binding of three BAP molecules at the active sites of JcPR-10a protein. JcPR-10a transgenics showed enhanced salt tolerance, as was evident by increased germination rate, shoot and root length, relative water content, proline, soluble sugar and amino acid content under salinity. Interestingly, the transgenics also showed enhanced endogenous cytokinin level as compared to WT, which, further increased with salinity. Exposure of gradual salinity resulted in increased stomatal conductance, water use efficiency, photosynthesis rate and reduced transpiration rate. Furthermore, the transgenics also showed enhanced resistance against *Macrophomina* fungus. Thus, JcPR-10a might be working in co-ordination with cytokinin signalling in mitigating the stress induced damage by regulating different stress signalling pathways, leading to enhanced stress tolerance.

**Keywords:** cytokinin, docking, JcPR-10a, *Macrophomina phaseolina*, photosynthesis, salinity, tobacco, transgenics

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