JOINT EVENT

20th Global Congress on Biotechnology

&

3rd International Conference on Enzymology and Molecular Biology

March 05-07, 2018 London, UK

Scientific Tracks & Abstracts

Day 1

Sessions:

Day 1 March 05, 2018

Industrial Biotechnology | Pharmaceutical Biotechnology | Biotechnology Applications

Session Chair
Sergey Suchkov
Moscow State Medical University, Russia

Session Introduction

Title: **Efficiency of the antioxidative system is the first prerequisite for effective doubled haploids production with the use of isolated microspore culture method**

Iwona Zur, Polish Academy of Sciences, Poland

Title: **Development of approach to obtain *Brachypodium distachyon* L. regenerative plants with morphogenetic stability**

Omirbekova N Zh, Al-Farabi Kazakh National University, Kazakhstan

Title: **Toxicological aspects of physiological and biochemical changes with potassium silicate and silica nano-particles on albino rat**

Helmi Mohamed El-bendary, Fayoum University, Egypt

Title: **Engineering carbon-conserving synthetic pathways for assimilation and conversion of C5/C6 carbon sources into added value chemicals**

Jean M Francois, Université Fédérale Toulouse Midi-Pyrénées, France

Iwona Żur et al., J Biotechnol Biomater 2018, Volume 8
DOI: 10.4172/2155-952X-C2-091

Efficiency of the antioxidative system is the first prerequisite for effective doubled haploids production with the use of isolated microspore culture method

Iwona Żur[1], Jozsef Fodor[2], Katarzyna Gawrońska[3], Gabriela Gołębiowska-Pikania[3], Katarzyna Juzoń[1], Przemysław Kopeć[1], Ewa Surówka[1], Franciszek Janowiak[1], Monika Krzewska[1], Ewa Dubas[1] and **Balazs Barna[2]**

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The technology of doubled haploids as the fastest route to total homozygosity is highly appreciated in many domains of basic research and breeding. Among several methods, the one using isolated and *in vitro*-cultured immature cells of male gametophyte induced towards embryogenic development (microspore embryogenesis-ME) possesses the highest potential for commercial application. However, efficient ME induction requires a precisely balanced stress treatment, strong enough to induce microspore reprogramming but not exceeding cell stress tolerance threshold. As the general cause of injuries in *in vitro*-cultured cells is the overproduction of reactive oxygen species (ROS), an efficient antioxidative defence was suggested as the first prerequisite for stress survival and effective ME initiation. To establish the role of ROS and the antioxidative system in ME initiation, the generation of hydrogen peroxide, and the activities of antioxidative enzymes and low molecular weight antioxidants were analysed in isolated microspores of two cultivars of barley (Hordeum vulgare L.), winter cv. Igri and spring cv. Golden Promise, differing significantly with respect to embryogenic potential. The analyses were conducted in microspores redirected towards embryogenic development by low temperature tillers pre-treatment (4 weeks at 4°C). Additionally, the effects of compounds known as cellular redox status modifiers, e.g. glutathione and L-2-oxo-4-thiazolidinecarboxylic acid (OTC), on microspore viability and ME initiation efficiency were estimated. The received results suggest that the activity of the antioxidative system is the first prerequisite for successful ME initiation, though in the case of its low activity, antioxidative defence could be supported by the application of exogenous antioxidants.

Biography

Iwona Żur completed her PhD and Habilitation in the field of Agronomy and Plant Physiology at the University of Agriculture in Kraków, Poland. Since 2010, she has been the Head of the Department of Cell Biology at the Institute of Plant Physiology Polish Academy of Sciences. She has published 38 papers in peer-reviewed journals.

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Notes:

Development of approach to obtain *Brachypodium distachyon* L. regenerative plants with morphogenetic stability

Omirbekova N Zh, Mursalieva V K, Zhusupova A I, Zhunusbaeva Zh K and **Kenzhebaeva S S**
Al-Farabi Kazakh National University, Kazakhstan

The aim of the research is development of effective methodological approaches of *in vitro* cultivation, object 21 line (BD21) B. distachyon. In order to develop cultivation methods, ability for callus formation, regeneration of generative and vegetative organs of VD21 was studied. To cultivate, Linsmayer-Skoog and Murashige-Skoog medium, additional introduction of phytohormones was used. Aseptic culture conditions for callusogenesis cultivation: under dark conditions at a temperature of 24°C, for t shoots regeneration: 16/8 hour photoperiod and lighting of 3000 lux. Inflorescence and immature embryos isolated from green spikes of vegetating plants and isolated embryos from mature seeds were used as primary explants to induce callus formation *in vitro*. During immature embryo cultivation, callus formation takes place near the corimbe for 20-25 days. During the cultivation of whole caryopsis with mature embryos, the sprouts grew after a week of cultivation on MS medium without hormones. The level of maturity of isolated caryopsis has a significant influence on the callus formation and the type of callus tissue. The mature caryopsis formed callus on the 10th day of cultivation with a frequency of 75%. The cultivation of the overgrown caryopsis in the dark on medium MS 1 with 2 mg/L 2.4 DPA, led to the formation of a primary shoot in 60% of explants; the formation of callus in the area of the scute, but for 30-35 days. Passage of the callus on the same medium and on the hormone-free medium led to the appearance of greenish pointwise impregnation of 30% of the calluses. For microclonal propagation, nodal segments of young shoots of plants were introduced into the culture. To culture introduction, side shoots 5 cm long with 3-4 interstitial sites were cut, the microcrops were planted in inducing media. The shoot-forming capacity of primary explants was about 59%; the multiplication factor for two passages was 5.7.

Recent Publications

1. Omirbekova N, Kenzhebayeva S, Capstaff N, Fatma Sarsu, et al. (2017) Searching a spring wheat mutation resource for correlations between yield, grain size, and quality parameters. Journal of Crop Improvement 31:209-228.
2. Omirbekova N, Kenzhebayeva S, Doktyrbay G et al. (2016) Frequency of vernalization requirement associated dominant VRN-A1 gene and earliness related Esp-A1 candidate genes in advanced wheat mutant lines and effect of allele on flowering time. International Journal of Biology and Chemistry 9:24-30.
3. Omirbekova N, Zhussupova A and Zhunusbayeva Zh (2015) *Brachypodium distachyon* as a model plant in wheat rust research. International Journal of Biology and Chemistry 2:52-55.

Biography

Omirbekova N Zh graduated from Al-Farabi Kazakh National University and Lomonosov Moscow State University and has completed her Doctoral studies from Al-Farabi Kazakh National University. She is currently a Professor at the Department of Molecular Biology and Genetics, School of Biology and Biotechnology of KazNU named after Al-Farabi (Republic of Kazakhstan). Her research interests include chemical mutagenesis, genetics and biochemistry of wheat. She has published more than 30 papers in high valued journals.

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Notes:

Helmi Mohamed El-bendary, J Biotechnol Biomater 2018, Volume 8
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Toxicological aspects of physiological and biochemical changes with potassium silicate and silica nano-particles on albino rat

Helmi Mohamed El-bendary
Fayoum University, Egypt

Naturally occurring micron sized silica has gained enormous popularity as a physically active insecticide. Nano-sized silica has insecticidal properties and would be needed in lesser quantity in comparison with conventional insecticides because of the huge surface to volume ratio of nanoparticles. Nano molecules have been widely used in consumer and industrial applications, such as medicine, cosmetics and foods, because they exhibit unique physicochemical properties and innovative functions. However, nanomaterials (NMs) can also be problematic in terms of eliciting a toxicological effect by their small size. The present study was designed to examine the toxic effects of orally administered pesticide Sil-MATRIX 29% (potassium silicate) and silica nano-particles (SiO_2-NPs) using male albino rats, at sublethal doses [2/5, 1/4 and 1/8 LD50], relative to control on [body, organs weight such as liver, kidney, heart, spleen, and cytotoxic effect (such as total protein content levels as biochemical aspects)] for 28 and 45 days' time exposure period. Orally ingested Sil-MATRIX 29% and silica nanoparticles (SiO_2-NPs) [2/5, 1/4 and 1/8 LD50] were not associated with significant changes in the average gain of body and organ weight. On the other hand, total protein content value after ingestion with Sil-MATRIX and SiO_2-NPs for all doses and treatments time period were increased significantly in a pattern similar to control rats. Our results suggested that the well-dispersed nano-silica cytotoxic effect caused systemic exposure in mouse and induced mutagenic activity. Our information indicated that further studies of relation between physicochemical properties and biological responses are needed for the development and safer form of (NMs).

Biography

Helmi Mohamed El-bendary is Assistant Professor of Agriculture at Fayoum University. He finished his BSc in Plant Protection Department at Cairo University and D.S.P.U at Mediterranean Agronomic Institute, Greece, M.S.c. at Cairo University, LLB at Cairo University, and Ph.D. at Mansoura University.

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Notes:

Jean M François et al., J Biotechnol Biomater 2018, Volume 8
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Engineering carbon-conserving synthetic pathways for assimilation and conversion of C5/C6 carbon sources into added value chemicals

Jean M François[1,2], **Ceren Alkim**[1,2] and **Thomas Walther**[1,3]
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[2]Toulouse White Biotechnology, France
[3]Technische Universität Dresden, Germany

The development of carbon efficient pathways for added value (bio)chemicals production is the essence of White Biotechnology. The limit of carbon conservation in all (bio)chemical syntheses is determined by the electron balance in substrate(s) and product(s). 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 rely 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(Khk-C) and human aldolase (Aldo-B). Then glycoaldehyde can be either reduced by endogenous aldehyde reductase to produce ethylene (EG) 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. 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. We then 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.

Biography

Jean M François got his PhD in Biological Science and Agronomy from the University of Louvain (Belgium) in 1988. He is Professor of Industrial Microbiology and BioNanotechnology at the Federal University of Toulouse, School of Engineer. His research activity concerns integrated physiology and functional genomics in microbial systems, with a specific focus on carbon and energy metabolism in yeast and filamentous fungi . He is author of more than 180 papers and 15 patents and Editor in Chief of BMC Biotechnology for Biofuels.

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Notes:

Sessions:

Structural Enzymology | Enzymology & Biochemistry

Session Chair
Magali Remaud-Simeon
INSA-Tolouse, France

Session Introduction

Title: **Molecular enzymology of DNA methyltransferases – conformational changes and allosteric regulation**
Albert Jeltsch, University Stuttgart, Germany

Title: **N-acyltransferases and their role in fatty acid amide biosynthesis**
David J Merkler, University of South Florida, USA

Title: **Lysyl oxidase: A versatile and elusive enzyme**
Karlo M Lopez, California State University-Bakersfield, USA

Title: **ProxiMAX randomization: Precision protein engineering**
Anna V Hine, Aston University, UK

Title: **Stability and function of a thermophilic cytochrome c'**
Sotaro Fujii, Hiroshima University, Japan

Albert Jeltsch, J Biotechnol Biomater 2018, Volume 8
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Molecular enzymology of DNA methyltransferases – conformational changes and allosteric regulation

Albert Jeltsch
University Stuttgart, Germany

DNA methylation is an essential epigenetic chromatin modification. The setup and maintenance of DNA methylation patterns depends on the coordinated activity of DNA methyltransferases (DNMTs) and their allosteric regulation by interacting proteins, other chromatin modifications and post-translational modifications. I will present novel assays for DNMTs including single enzyme assays to study their mechanism and conformationally locked mutants to study allosteric effects. Based on this, recent data regarding the regulation and targeting of DNMTs by allosteric effect will be presented. Moreover, I will present insights into the mechanism of DNMTs regarding target site location, specificity and processivity.

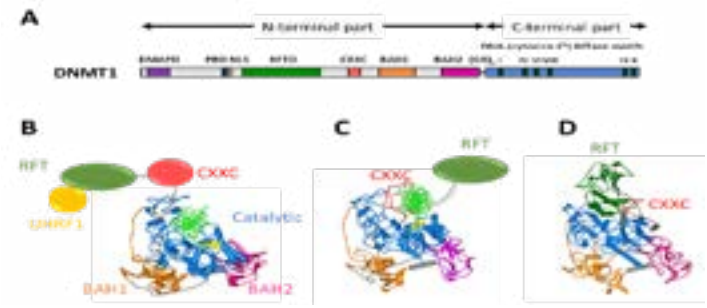

Figure 1: Domain structure and 3D structure of DNMT1. A) Domain structure of DNMT1 (for descriptions of the domains refer to the main text). B-D) Different structures of DNMT1 with AdoHcy shown in yellow and DNA in light green. B) Structure with DNA bound in the active site. UHRF1 (yellow) stabilizes the active conformation of DNMT1. C) Structure with unmethylated DNA bound to the CXXC domain. D) Apoenzyme structure with the RFT domain blocking of the active site.

Recent Publications

1. Lungu et al. (2017) Modular fluorescence complementation sensors for live cell detection of epigenetic signals at endogenous genomic sites. Nature Communications 8:649.
2. Maier, Möhrle and Jeltsch (2017) Design of synthetic epigenetic circuits exhibiting positive feedback, memory effects and reversible switching, Nature Communications 8:15336.
3. Jurkowska and Jeltsch (2016) Allosteric control of mammalian DNA methyltransferases - a new regulatory paradigm. Nucleic Acids Res. 44:8556-8575.
4. Bashtrykov, et al. (2014) The UHRF1 protein stimulates the activity and specificity of the maintenance DNA methyltransferase DNMT1 by an allosteric mechanism. J. Biol. Chem. 289:4106-15.
5. Jeltsch and Jurkowska (2014) New concepts in DNA methylation. Trends Biochem Sci. 39:310-18.

Biography

Albert Jeltsch completed his PhD working on the mechanism of restriction endonucleases at University of Hannover in 1994. Afterwards, he started to study DNA methyltransferases at Justus-Liebig University Giessen and at Jacobs University Bremen. Since 2011, he is a Professor of Biochemistry at the University Stuttgart. He received the Gerhard-Hess award (DFG) and BioFuture award (BMBF). He has long standing expertise in Biochemical study of DNA and protein methyltransferases, methyl lysine reading domains and in rational and evolutionary protein design. His work has been published in more than 250 publications in peer reviewed journals and he is in the editorial boards of several journals.

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David J Merkler, J Biotechnol Biomater 2018, Volume 8
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N-acyltransferases and their role in fatty acid amide biosynthesis

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Fatty acid amides are a family of cell signaling lipids with the general structure of R-CO-NH-Y. This structural simplicity belies a wealth of diversity amongst this lipid family as the R-group is derived from fatty acids (R-COOH) and the Y-group is derived from biogenic amines (H2N-Y). The fatty acid amide family is divided into classes, defined by parent amines. Examples include the N-acylethanolamines (NAEs, R-CO-NH-CH2-CH2OH) and the N-acylglycines (NAGs, R-CO-NH-CH2-COOH). Other classes of fatty acid amides are known. The best known fatty acid amide is N-arachidonoylethanolamine (anandamide), a fatty acid amide found in the human brain that binds to the cannabinoid receptors. We have a long interest in the enzymes of fatty acid amide biosynthesis. We identified an enzyme that oxidizes the NAGs to the primary fatty acid amides and showed that inhibiting this enzyme led to the cellular accumulation of the NAGs. We have characterized several insect N-acyltransferases (from *D. melanogaster, B. mori,* and T. castaneum) that catalyze the acyl-CoA-dependent formation of fatty acid amides from an amine acyl-acceptor substrate. Knock-out experiments in *D. melanogaster* validate our *in vitro* substrate specific studies demonstrating that one novel N-acyltransferases, arylalkyl N-acyltransferase-like 2 (AANATL2), does catalyze the formation of N-acyldopamines *in vivo*. We developed a straightforward platform technology to rapidly identify substrates for our panel of uncharacterized insect N-acyltransferases. Our application of this technology leads to identification of an enzyme in *D. melanogaster*, agmatine N-acetyltransferase (AgmNAT), which catalyzes the formation of N-acetylagmatine, a virtually unknown metabolite. We have determined the X-ray structure of AgmNAT. Our work on AgmNAT hints at an unknown reaction in arginine metabolism and points to a novel class on fatty acid amides, the N-acylagmatine. The presentation will also include our results on the kinetic and chemical mechanisms of the novel N-acyltransferases.

Recent Publications

1. Dempsey D R et al. (2017) Structural and mechanistic analysis of Drosophila melanogaster agmatine N-acetyltransferase, an enzyme that catalyzes the formation of N-acetylagmatine. Sci. Rep. 7(1):13432.
2. Aboalroub A A et al. (2017) Acetyl group coordinated progression through the catalytic cycle of an arylalkylamine N-acetyltransferase. PLoS One. 12(5):e0177270.
3. Jeffries K A et al. (2016) Glycine N-acyltransferase-like 3 is responsible for long-chain N-acylglycine formation in N18TG2 cells. J. Lip. Res. 57(5):781-790.
4. Dempsey D R, Carpenter A M, Rodriguez Ospina S and Merkler D J (2015) Probing the chemical mechanism and critical regulatory amino acid residues in of Drosophila melanogaster arylalkylamine N-acyltransferase like 2. Insect Biochem. Mol. Biol. 66:1-12.
5. Dempsey D R et al. (2015) Mechanistic and structural analysis of a Drosophila melanogaster enzyme, arylalkylamine N-acetyltransferase like 7, an enzyme that catalyzes the formation of N-acetylarylalkylamides and N-acetylhistamine. Biochemistry. 54(16):2644-2658.

Biography

David J Merkler obtained a PhD in Biochemistry from Pennsylvania State University in 1985 and completed Postdoctoral Fellowships in Enzymology at Temple University School of Medicine (1985-1987) and the Albert Einstein College of Medicine (1987-1989). His next position was as Senior Scientist at Unigene Laboratories, Inc. involved in the *in vitro* production of a peptide hormone, calcitonin. In 1995, he moved back to academia as a Professor of Chemistry and Biochemistry first at Duquesne University (1995-1999) and then the University of South Florida (1999-present). His laboratory has been interested in the fatty amides: identification and characterization of the fatty acid amides (Lipidomics), identification and characterization of the enzymes of fatty acid amide biosynthesis (Enzymology and Structural Biology), and changes in the fatty acid amidome after targeted enzyme knock-out (subtraction lipidomics).

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Karlo M Lopez, J Biotechnol Biomater 2018, Volume 8
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Lysyl oxidase: A versatile and elusive enzyme

Karlo M Lopez
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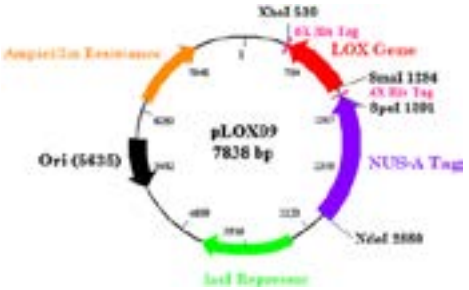
Lysyl oxidase is an extracellular matrix, copper-dependent, amine oxidase that catalyzes a key crosslinking step in collagen and elastin. The enzyme is synthesized as a proenzyme that, upon excretion to the extracellular matrix, is cleaved at the Gly168-Asp169 bond by procollagen C-proteinase in the mammalian form of the enzyme. Lysyl oxidase is highly regulated and changes in its regulation have been shown to play a role in fibrosis and several other diseases. More recently, the enzyme has been shown to play a paradoxical role in cancer. In the early stages of cancer, the cleaved pro-peptide has been shown to inhibit the RAS oncogene, whereas in late stages of cancer lysyl oxidase has been shown to promote metastasis. Lysyl oxidase is highly insoluble and this has hampered its full characterization. Recent work in the by our study group has addressed some of the issues associated with the insolubility and characterization of the enzyme. In particular, this talk will address how plasmids were used to increase enzyme yields over those obtained directly from bovine aortic tissue, the role solubility tags play on enzyme activity and suitability for characterization studies, and will end with an innovative new approach to drug delivery that targets lysyl oxidase in cancer cells but remains inactive in normal cells.



Recent Publications

1. Oldfield R, Johnston K, Limones J, Ghilarducci C and Lopez K (2017) Identification of histidine 303 as the catalytic base of lysyl oxidase via site – directed mutagenesis. The Protein Journal, doi: 10.1007/s10930-017-9749-3.
2. Smith M A, Gonzalez J, Hussain A, Oldfield R N, Johnston K A, et al. (2016) Overexpression of soluble recombinant human lysyl oxidase by using solubility tags: effects on activity and solubility. Enzyme Research 2016:1-7.
3. Lopez K and Greenaway F T (2011) Identification of the copper-binding ligands of lysyl oxidase. Journal of Neural Transmission 118:1101-1109.
4. Herwald S, Greenaway F and Lopez K (2010) Purification of high yields of catalytically active lysyl oxidase directly from Escherichia coli cell culture. Protein Expression and Purification 74:116-121.

Biography

Karlo M Lopez is currently an Associate Professor of Biochemistry at California State University, Bakersfield. He received a PhD from Clark University and was a Howard Medical Institute Fellow at Pomona College. His research focuses primarily on the structural characterization of lysyl oxidase and understanding the role this enzyme plays in cancer metastasis. He is a member of the Committee on Ethics of the American Chemical Society and was part of the Task Force for Safety Education Guidelines.

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Notes:

Anna V Hine, J Biotechnol Biomater 2018, Volume 8
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ProxiMAX randomization: Precision protein engineering

Anna V Hine
Aston University, UK

ProxiMAX randomization is the technology that lies behind Isogenica's Colibra™ offering. It is a defined saturation mutagenesis process that delivers precision control of both identity and relative ratio of amino acids at specified locations within a protein/antibody library. Thus unwanted amino acids such as cysteine and methionine can be eliminated from libraries because no constraints are imposed by the genetic code. Moreover, the process is non-degenerate, which means that encoding DNA libraries are as small as is physically possible. ProxiMAX relies on a process of saturation cycling comprising ligation, amplification and digestion for each cycle and is the science behind the commercial Colibra™ technology. Currently focused on antibody libraries but with achieved diversities of >99% (6 & 11 saturated codons) and the potential to generate libraries of up to 10^{14} components, we contest that ProxiMAX randomization is a vital tool in engineering any protein library of the highest quality. This presentation will examine the development of the ProxiMAX process and give examples of libraries created to date.

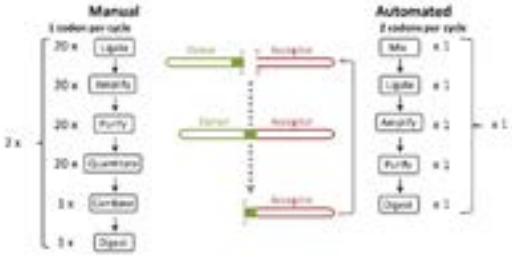

Figure 1: Overview of the ProxiMAX

Recent Publications

1. Ferreira Amaral M M, Frigotto L and Hine A V (2017) Beyond the natural proteome: nondegenerate saturation mutagenesis - methodologies and advantages. Meth. Enyzmol. 585:111-133.
2. Frigotto L, Smith M E, Brankin C, Sedani A, Cooper S E, Kanwar N, Evans D, Svobodova S, Baar C, Glanville J, Ullman C G and Hine A V (2015) Codon-precise, synthetic, antibody fragment libraries built using automated hexamer codon additions and validated through next generation sequencing. Antibodies 4:88-102.
3. Chimonides G F, Behrendt J M, Chundoo E, Bland C, Hine A V, Devitt A, Nagel D A and Sutherland A J (2014) Cellular uptake of ribonuclease A functionalised core–shell silica microspheres. J Mater Chem B, 2:7307-7315.
4. Nagel D, Behrendt J M, Chimonides G F, Torr E E, Devitt A, Sutherland A J and Hine A V (2014) Polymeric microspheres as protein transduction reagents. Mol. Cell Proteomics, 13:1543-1551.
5. Ashraf M, Frigotto L, Smith M E, Patel S, Hughes M D, Poole A J, Hebaishi H R M, Ullman C G and Hine A V (2013) ProxiMAX randomisation: a new technology for non-degenerate saturation mutagenesis of contiguous codons. Biochem. Soc. Trans. 41:1189-1194.

Biography

Anna V Hine studied at the University of Manchester (UK) and Harvard Medical School. She is a Reader and Associate Dean Enterprise at Aston University (UK). In March 2013, she was named BBSRC Commercial Innovator of the Year 2013, for her work in transferring ProxiMAX randomization into SME Isogenica Ltd. She is a Molecular Biologist by training, she relishes interdisciplinary work.

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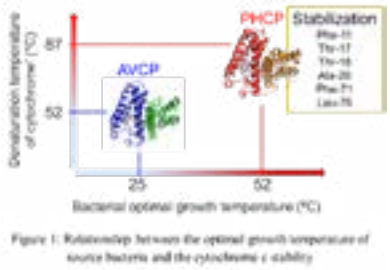
Stability and function of a thermophilic cytochrome c'

Sotaro Fujii and **Yoshihiro Sambongi**
Hiroshima University, Japan

Cytochromes c' are classified as heme proteins found in restricted Gram-negative bacteria. They usually form a homo dimeric structure, and the single subunit typically consists of four helix bundle. Biochemical analysis showed that they can bind diatomic gasses such as NO or CO, but not O_2. Recently we purified cytochrome c' from thermophilic *Hydrogenophilus thermoluteolus*, and named it PHCP. *H. thermoluteolus* grows optimally at 52°C, indicating that PHCP is more stable than homologous proteins from mesophiles. In this study, we compared stability and function of PHCP with its mesophilic homologue, *Allochromatium vinosum* cytochrome c' (AVCP) having 55 % amino acid sequence identity. In order to check the stability, we measured the circular dichroism spectra with increasing temperature. The denaturation temperature of PHCP was 87°C, which was higher than that of AVCP (52°C). The X-ray structure comparison between PHCP and AVCP revealed that the stability difference was due to the heme-related interactions and subunit-subunit interactions, which was also proofed by mutagenesis study. These results indicated that PHCP advantageously retains the native structure at high temperature. The PHCP X-ray structure further revealed a ligand binding channel and a penta-coordinated heme, as observed in the AVCP protein, indicating PHCP could bind diatomic gasses at high temperature. Thus, we measured the gas binding affinity of PHCP and AVCP using absorption spectra. The association constant (Ka) of PHCP with CO was 3 times lower than that of AVCP at 25°C, and PHCP could maintain normal spectral changes up to 60°C. In AVCP, such spectral changes with CO could not to be detected at 60°C, because of denaturation of AVCP. In conclusion, PHCP has a structure fulfilling the requirement for both gas-binding function and thermal stability. This stable cytochrome c' will become a model for protein engineering field.



Recent Publications

1. Fujii S, Masanari M, Inoue H, Yamanaka M, Wakai S, Nishihara H, Sambongi Y (2013) High thermal stability and unique trimer formation of cytochrome c' from thermophilic *Hydrogenophilus thermoluteolus*. Biosci Biotechnol Biochem 77:1677-1681.
2. Fujii S, Masanari M, Yamanaka M, Wakai S, Sambongi Y (2014) High stability of apo-cytochrome c' from thermophilic Hydrogenophilus thermoluteolus. Biosci Biotechnol Biochem 78:1191-1194.
3. Kato Y, Fujii S, Kuribayashi TA, Masanari M, Sambongi Y (2015) Thermal stability of cytochrome c' from mesophilic Shewanella amazonensis. Biosci Biotechnol Biochem. 80: 2365-2370.
4. Fujii S, Oki H, Kawahara K, Yamane D, Yamanaka M, Maruno T, Kobayashi Y, Masanari M, Wakai S, Nishihara H, Ohkubo T, Sambongi Y (2017) Structural and functional insights into thermally stable cytochrome c' from a thermophile. Protein Sci. 26: 737-748.

Biography

Sotaro Fujii is working on the stability, structure, and function of proteins that are important for microbial energy metabolism. A characteristic aspect of his research activity is comparison of the homologous proteins isolated from microorganisms living in extreme environments in which humans cannot live and those isolated from 'normal' environments.

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JOINT EVENT

20th Global Congress on Biotechnology

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3rd International Conference on Enzymology and Molecular Biology

March 05-07, 2018 London, UK

Scientific Tracks & Abstracts

Day 2

Sessions:

Molecular Enzymology | Enzyme Therapeutics | Enzymology in drug discovery | Industrial Biotechnology | Biotechnology Applications

Session Chair
Jennifer A Littlechild
University of Exeter, UK

Session Co-chair
Shree Kumar Apte
Bhabha Atomic Research Centre, India

Session Introduction

Title: **Engineering cyanobacterial nitrogen bio-fertilizer for rice cultivation in stressful environment**
Shree Kumar Apte, Bhabha Atomic Research Centre, India

Title: **The dual role of integrase in HIV-1 replication**
Mamuka Kvaratskhelia, University of Colorado School of Medicine, USA

Title: **Characterization of cystathionine γ-lyase from *T. gondii*: A target for drug development?**
Alessandra Astegno, University of Verona, Italy

Title: **Infulence of four kinds of additives and concentration on oats silage effect**
Yungui Yang, Northwest A & F University, China

Title: **Enhanced production of *Bacillus thuringiensis* subspecies israelensis delta endotoxin by the use of rotten pineapple juice and fish-amino acid as medium ingredients**
C Gopinathan, University of calicut, India

Shree Kumar Apte, J Biotechnol Biomater 2018, Volume 8
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Engineering cyanobacterial nitrogen bio-fertilizer for rice cultivation in stressful environment

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As a naturally abundant, photosynthetic, nitrogen-fixing microbe, the cyanobacterium *Anabaena* contributes significantly to the nitrogen and carbon economy of tropical soils, especially in cultivation of rice paddy. However, its nitrogen bio-fertilizer potential is adversely affected by common abiotic stresses. Engineering enhanced nitrogen fixation and stress tolerance capabilities in this microbe through genetic manipulation is seriously limited due to the unavailability of appropriate tools and techniques and knowledge of suitable candidate genes. In recent years, our laboratory has devised an electroporation protocol for genetic transformation that achieves high frequency gene transfer and overcomes problems associated with the current practice of tri-parental conjugation between *E. coli* strains and *Anabaena*. We have also constructed (a) a suitable vector for new gene discoveries, and (b) a novel integrative expression vector pFPN, placing desired genes at a defined locus in *Anabaena* genome and facilitates their high level expression from an eco-friendly light-inducible promoter. Using these tools we have identified several genes responsible for enhanced heterocyst formation and nitrogen fixaton (hetR), chaperones (groESL, cpn60) for protein folding and homeostasis, and several oxidative stress tolerance genes (superoxide dismutases, catalases and peroxiredoxins) which confer superior stress tolerance to *Anabaena*. The approach has proved very useful for constructing recombinant *Anabaena* strains capable of nitrogen fixation in stressful environments.

Biography

Shree Kumar Apte obtained his Master's in Botany from Jiwaji University, Gwalior, India with a Gold Medal in Science Faculty in 1972. He researched at the Bhabha Atomic Research Centre (BARC), Mumbai, India for 42 year, before retiring in 2014 as a distinguished Scientist and Director of the Bioscience Group, BARC. He is an elected fellow of all three National Science Academies and the National Agriculture Science Academy in India. Currently he serves as Emeritus Professor, Homi Bhabha National Institute, J C Bose National Fellow (DST) and Raja Ramanna Fellow (DAE) at BARC, Mumbai.

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Mamuka Kvaratskhelia, J Biotechnol Biomater 2018, Volume 8
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The dual role of integrase in HIV-1 replication

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A key HIV-1 enzyme integrase catalyzes irreversible insertion of a viral DNA copy of its RNA genome into human chromosome, which is essential for viral replication. Therefore, integrase is an important therapeutic target. Productive integration into host chromatin results in the formation of the strand transfer complex (STC) containing catalytically joined viral and target DNAs. We have used cryo-EM coupled with biochemistry and virology experiments to obtain high-resolution structures for STCs and to characterize the integrase multi-subunit assemblies into large, nucleoprotein complexes. We are currently extending these studies to elucidate structural basis for the mode of action of clinically used integrase strand transfer inhibitors (INSTIs), which bind to the enzyme active site in the context of the integrase-viral DNA complex and block the strand transfer reaction. Our parallel efforts are focused on studying allosteric HIV-1 integrase inhibitors (ALLINIs), which are currently undergoing clinical trials (2-5). Unlike INSTIs, ALLINIs bind at the integrase dimer interface and induce aberrant protein multimerization. Unexpectedly, in infected cells ALLINIs were significantly more potent during virion maturation rather than during integration. ALLINIs markedly altered virus particle morphogenesis by misplacing the ribonucleoprotein complexes outside the protective viral capsid shell and yielded inactive virions. In turn, these findings have suggested that integrase has a second function in HIV-1 biology. Our follow up studies have revealed that integrase directly binds the viral RNA genome in virions. These interactions have specificity, as integrase exhibits distinct preference for select viral RNA structural elements. ALLINIs impair integrase binding to viral RNA in virions of wild-type, but not escape mutant, virus. These results reveal an unexpected biological role of integrase binding to the viral RNA genome during virion morphogenesis and elucidate the mode of action of ALLINIs. Collectively our findings indicate that viral integrase plays a dual role during HIV-1 replication.

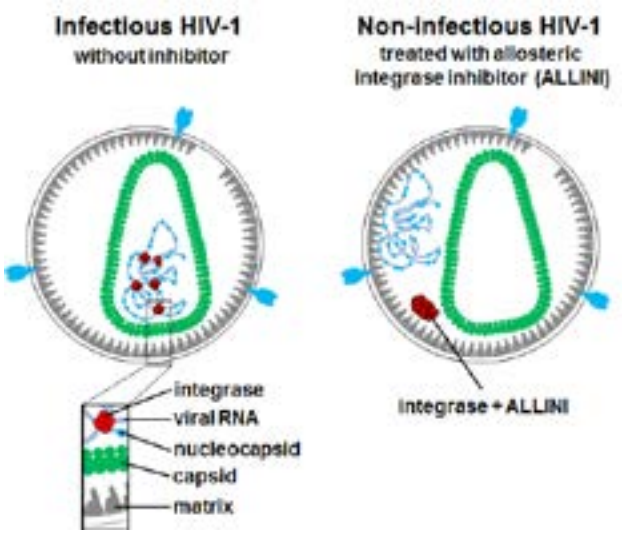

Figure 1: The schematic to show that HIV-1 integrase has a second, non-catalytic function in HIV-1 biology as it binds the viral RNA genome to promote particle maturation. ALLINI treatments mislocalize ribonucleoprotein complexes outside of the protective capsid core.

Recent Publications

1. Passos D O, Li M, Yang R, Rebensburg S V, Ghirlando R (2017) Cryo-EM structures and atomic model of the HIV-1 strand transfer complex intasome. Science. 355(6320):89-92.
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3. Sharma A, Slaughter A, Jena N, Feng L, Kessl J J (2014) A new class of multimerization selective inhibitors of HIV-1 integrase. PLoS Pathog. 10(5):e1004171.
4. Jurado K A, Wang H, Slaughter A, Feng L, Kessl J J et al. (2013) Allosteric integrase potency is determined through the inhibition of HIV-1 particle maturation. Proc. Natl. Acad. Sci. USA. 110(21):8690-8695.
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Biography

Mamuka Kvaratskhelia began his independent research career at the Ohio State University in 2003 and has focused on better understanding of the structure and function of HIV-1 integrase as a therapeutic target. He has recently (2017) moved to University of Colorado Denver as a Professor of Medicine (Infectious Diseases) to further extend his studies on HIV-1 integrase. By employing innovative biochemical, biophysical, structural biology, molecular biology and virology approaches, his research team has made many important contributions to the field, which include the discovery of second, non-catalytic role of integrase in HIV-1 biology and elucidating the mode of action of ALLINIs.

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Characterization of cystathionine γ-lyase from *T. gondii*: A target for drug development?

Alessandra Astegno
University of Verona, Italy

Toxoplasma gondii is a protozoan parasite of medical and veterinary relevance responsible for toxoplasmosis in humans. As there is currently no vaccine available for human, the identification of good target candidates for future drug development is urgently required. A recent proteomic analysis of partially sporulated oocysts of *T. gondii* showed that oocyctes have a greater capability of *de novo* amino acid biosynthesis, shedding light on a stage-specific subset of proteins whose functional profile is consistent with the oocyst need to resist various environmental stresses. Among these putative oocyst/sporozoite-specific proteins, three enzymes involved in cysteine metabolism, i.e., cystathionine β-synthase, cystathionine γ-lyase (CGL) and cysteine synthase, were found. However, despite the central metabolic roles of these enzymes, the functionality of none of them has so far been investigated. Herein, CGL from *T. gondii* (TgCGL) has been cloned, expressed and physiochemically and enzymatically characterized. The purified TgCGL is a functional enzyme which splits L-cystathionine almost exclusively at the CγS bond to yield L-cysteine. This finding likely implies that the reverse transsulfuration pathway is operative in the parasite. The enzyme displays only marginal reactivity toward L-cysteine, which is also a mixed-type inhibitor of TgCGL activity, therefore indicating a tight regulation of cysteine intracellular levels in the parasite. Structure-guided homology modelling revealed two striking amino acid differences between human and parasite CGL active sites (Glu59 and Ser340 in human to Ser77 and Asn360 in toxoplasma). Mutation of these two residues to the corresponding residues in human revealed their importance in modulating both substrate and reaction specificity of the parasitic enzyme. Our findings might have far-reaching implications for the use of TgCGL as anti-toxoplasmosis drug target.

Recent Publications

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4. Rossignoli G, Phillips R S, Astegno A, Menegazzi M, Voltattorni CB, et al. (2018) Phosphorylation of pyridoxal 5'-phosphate enzymes: an intriguing and neglected topic. Amino Acids. 50(2):205-215.
5. Allegrini A, Astegno A, La Verde V and Dominici P (2017) Characterization of C-S lyase from *Lactobacillus delbrueckii subsp. bulgaricus* ATCC BAA-365 and its potential role in food flavour applications. J Biochem. 161(4):349-360.

Biography

Alessandra Astegno is interested in different aspects of Protein Chemistry and Enzymology, including folding, evolution and structure-function relationship of proteins and macromolecular assemblies. She is currently an Assistant Professor in Biochemistry at the Department of Biotechnology of the University of Verona. She has a solid background in recombinant protein expression and purification, functional and structural characterization of pyridoxal phosphate-dependent enzymes as well as metallo-proteins.

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Influence of four kinds of additives and concentration on oats silage effect

Yungui Yang, Yafei Li, Ting Guo, Yunqing Huang and **Wei Li**
Northwest A & F University, China

Statement of the Problem: The aim for this research was to study the effect of different additives and concentration on oat silage. Selected Dancer as material was used for silage in milk stage, and four kinds of additives respectively at different concentration were used. They were lactobacillus (0 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg), formic acid (0 ml/kg, 1 ml/kg, 5 ml/kg, 10 ml/kg), sucrose (0%, 1%, 2%, 4%) and cellulose (0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg). The materials were ensiled at room temperature and opened 60 days later, and the fermentation quality and the chemical composition were analyzed. Results showed that it had a positive impact on Dancer silage with four kinds of additives. Considering nutritional value index(crude protein, ether extract and crude fiber) When cellulase were applied at 50 mg/kg, the oats silage were excellent. Considering silage quality indexes of pH value, AN/TN, soluble sugar content and lactic acid content, sucrose at 2% level was the best concentration. The best concentration of different additives were that: lactobacillus (5 mg/kg), formic acid (5ml/kg), sucrose (2%) and the influence on silage quality have no close connection with concentration when added cellulase.

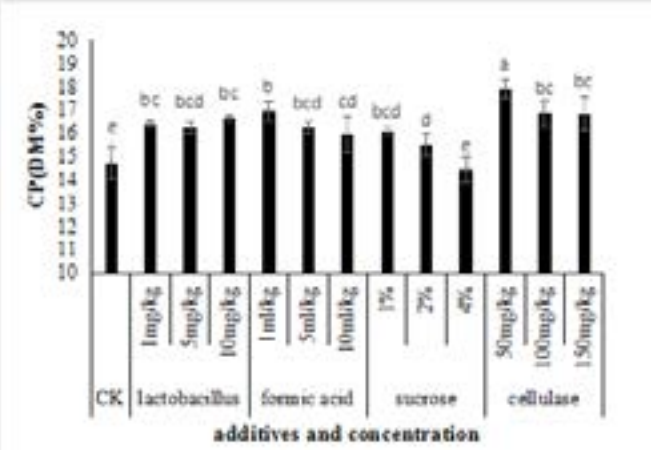

Fig. 1 Percent CP of oat as affected by four additives

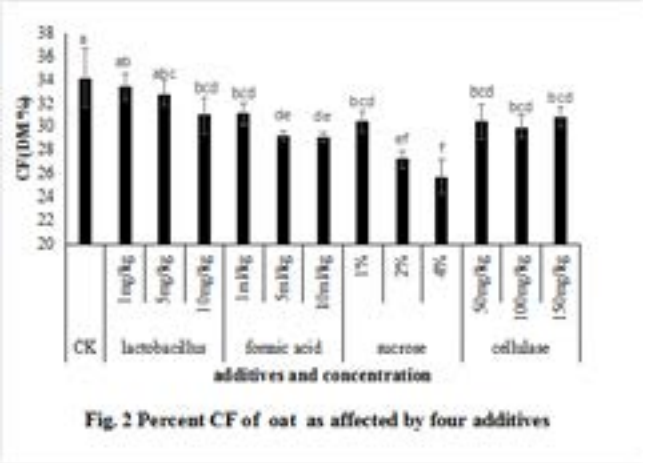

Fig. 2 Percent CF of oat as affected by four additives

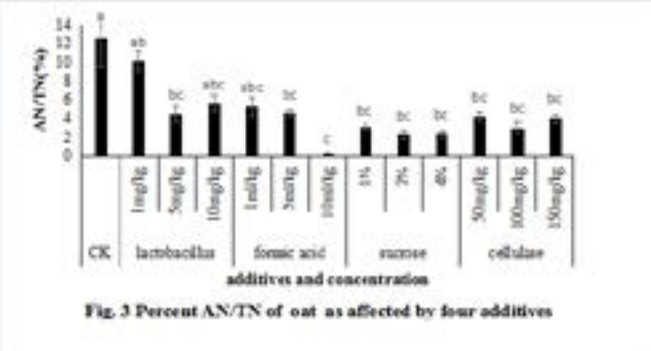

Fig. 3 Percent AN/TN of oat as affected by four additives

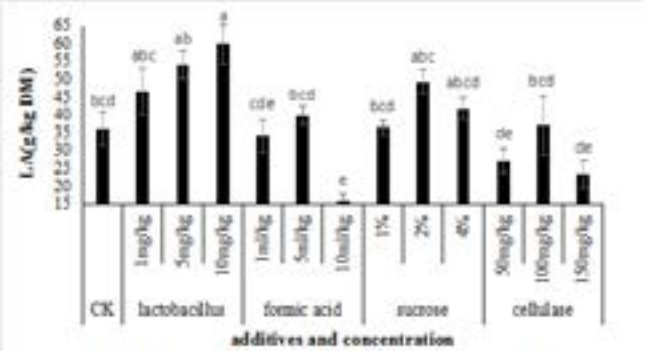

Fig. 4 Percent LA of oat as affected by four additives

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1. Ni K, Wang F, Zhu B, et al. (2017) Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. Bioresource Technology. 238: 706-715.
2. Carvalho V V, Paulino M F, Detmann E, et al. (2017) Effects of supplements containing different additives on nutritional and productive performance of beef cattle grazing tropical grass. Tropical Animal Health and Production. 49(5): 983-988.
3. Wang S, Yuan X, Dong Z, et al. (2017) Characteristics of lactic acid bacteria isolated from different sources and their effects on the silage quality of oat (Avena sativa L.) straw on the Tibetan Plateau. Grassland Science. Vol(Issue): pg nos.
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5. Ning T, Wang H, Zheng M, et al. (2017) Effects of microbial enzymes on starch and hemicellulose degradation in total mixed ration silages. Asian-Australasian Journal of Animal Sciences. 30(2): 171.

Biography

Yungui Yang graduated from China Agricultural University in 1987 with a Master's degree in Grassland Science. In August of the same year, he taught at the Northwest A&F University. In 2006, he received a Doctorate in Soil Resources and Information Technology direction. Now he is a member of Lawn Professional Committee of China Grass Society and Director of Grassland Resources and Management Committee. In October 2012, he went to the United States to attend the international annual conference jointly organized by the American Society of Agricultural Sciences, the Crop Science Society and the Soil Science Society. In October 2013, he went to Mongolia to attend the Eurasian Pacific Union Symposium. He is currently a Member of Comprehensive Utilization of Straw Resources of China Agronomy, a review expert of Life Science Division of NSFC, a fellow of American Society of Agricultural Sciences, Soil Society and Crop Society.

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Enhanced production of *Bacillus thuringiensis* subspecies israelensis delta endotoxin by the use of rotten pineapple juice and fish-amino acid as medium ingredients

C Gopinathan and **Sonia Sharma**
University of Calicut, India

Mosquito borne diseases not only cause loss of lives but also impose heavy health and economic burdens. Extensive use of chemical insecticides for the control of malaria and other mosquito borne diseases has led to the development of resistance in mosquitoes to these insecticides and are hazardous to the environment. Biolarvicides of the strain *Bacillus thuringiensis* israelensis (Bti), serotype H-14 is highly effective against mosquito larvae. Even though Bti products are efficient controls for mosquito and black fly larvae, their use in developing countries is limited by their cost. Thus, there is a need to reduce the overall production cost of Bti in order to make it competitive in the market. It depends on many factors; however, the raw material cost is one of the most important criteria which may comprise >70% of the overall production cost. Fruit wastes are available in plenty and contain mainly fructose as the carbon source, which is easily fermentable and can substitute costly substrates like glucose. Channelizing huge quantities of rotten/ waste pineapples which otherwise are discarded can substantially reduce production cost of Bti. Similarly fish-amino acid produced by fermenting rotten fish and jaggery/molasess has proved to be excellent as a medium supplement; especially to overproduce the much wanted delta endotoxin produced by Bti. India is one of the countries leading in fruit and vegetable production. It is also blessed with one of the longest coastline in the world of approximately 7516.6 km. The total annual catch is around 4 million metric tons. In addition it is second after Brazil in sugarcane cultivation with an annual yield of 3412 million metric tons. The massive availability of fruit wastes (pineapples) and huge quantities of rotten /discarded fish , which are freely available, all can be channelized for cost effective production of this value added product, substantially lowering the media cost of Bti production when scale- up is attempted. Results show biomass increase of up to 27% compared to control when pineapple juice was used as the main carbon source. The toxicity improvements with fish-amino acid supplemented medium, shows considerable reduction in killing time of *Aedes aegypti* larvae.

Biography

C Gopinathan is working as a Associate Professor in the Department of Biotechnology at the University of Calicut. He has finished his MSc, MTech in Biotechnology. His specialization is towards Bioprocess Technology/Fermentation Technology. He is the former member of Academic Council, University of Calicut, American Society for Microbiology and Association of Microbiologists of India.

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