

CONTENTS

SECTION NAME	PAGE No
1. EDITOR'S MESSAGE	4
2. MESSAGE FROM PRESIDENT, PSI	5
3. PSI 2019, NDRI	6-14
4. HUPO 2019	<u>14-19</u>
5. RESEARCH	20-23
6. WORKSHOP	

PSI EXECUTIVE COMMITTEE

PRESIDENT Dr. Subhra Chakraborty

VICE PRESIDENT

Dr. Shantanu Sengupta Dr. Arun Bandyopadhyay

GEN. SECRETARY Dr. Srikanth Rapole

TREASURER Dr. Swasti Raychaudhuri

JT. SECRETARY Dr. Ashok Kr Mohanty

PAST PRESIDENT OF PSI

Dr. Ravi Sirdeshmukh (Founder President) Dr. Surekha Zingde Dr. Utpal Tatu

MEMBER

Dr. Utpal Tatu

- Dr. Ravi Sirdeshmukh
- Dr. Rakesh K Mishra
- Dr. Abhijit Chakrabarti
- Dr. Mahesh J Kulkarni
- Dr. K Dharmalingam
- Dr. Niranjan Chakraborty
- Dr. Debasis Dash
- Dr. Sanjeeva Srivastava
- Dr. Suman Kundu
- Dr. Tushar Kanti Maiti
- Dr. Krishnan Venkataraman
- Dr. Ramesh Ummanni
- Dr. K. Balamurugan
- Dr. Geetanjali Sachdeva

Dear PSI Members,

The last six months have been extremely busy for our EC members and the Organizing team of the 11th PSI Annual meeting which was held in Karnal in December 2019. This issue brings a detailed report prepared by Dr. Ashok Mohanty, Convener of the meeting. The Pre conference workshops, Education day and the Conference thereafter brought together over 450 participants from India and abroad who had the opportunity to interact with each other and the stalwarts in Proteomic science. I am sure many new collaborations would have been initiated at the meeting.

Besides the Annual meeting at Karnal, there was a school and a symposium focused on Advances in Biomedical Mass Spectrometry at Kolkatta. Dr. Soumen Manna,Convener of the program has provided a detailed report of the proceedings.

This year PSI supported five students to attend the HUPO 2019 Congress in Adelaide. The abstracts of their work are included in this issue. This is followed by summaries of some interesting recent research published by our members. It is to be noted that proteomic tools are being used in a variety of scientific areas of research in Institutions across the country. This is really very encouraging, as PSI has played a major role in promoting proteomic based research in India.

With best wishes,

Dr. Tushar Kanti Maiti tkmaiti@rcb.res.in Dr. Surekha M. Zingde surekha.zingde@gmail.com

Dear Members of the Proteomics Society, India,

My hearty welcome to the new PSI Executive Committee members. Next on behalf of the PSI-EC and my own, I would like to extend a warm welcome to all the members of Proteomics Society, India.

May I begin by acknowledging the work of the members of the past EC in pursuing the interests and offering their invaluable time and service to the Society. The present EC started functioning early 2020 with 21 members whose knowledge, interest and experience would immensely help furthering the activities of the Society in the coming years. The confidence reposed in the new team will be fully respected and protected.

As was intended, the Proteomics Society, India, has continued to be devoted and dedicated to provide an excellent platform for its members and all the proteomers from India and abroad. Each year, the Society endeavours to come up with select themes for its annual meeting focused upon contemporary issues in the field. Recent years have shown increased frequency of high complexity diseases worldwide. The main objective of 11th Annual meeting of PSI 2019 and International conference on "System Integrated Bio-Omics, One Health and Food Safety" (ICPBHF-2019) was to bring together leading scientists and scholars in the area, to discuss the practical challenges, concerns and strategies in the field, promote collaborations and information exchange and share the new findings. We all had a wonderful time at ICAR-NDRI, Karnal last December. Also, the school and the symposium on Advances in Biomedical Mass Spectrometry (SSABMS 2019) at SINP, Kolkata provided another platform for academic interaction. For the first time, five students could attend the HUPO 2019 World Congress at Adelaide, Australia with the combined support from PSI and the local organizers. Six other students and researchers from India were among the travel award winners from HUPO 2019 organizing committee to attend the meeting.

I am delighted to share with you that India would be hosting the HUPO Congress soon. We shall be updating you time to time the progress and new happenings towards holding the meeting. If you have not done so already, it is time to visit <u>https://hupo2020.org/</u> and PSI website to know more about this years HUPO connect meeting and the annual meeting of PSI.

It gives me great pleasure to note that an exciting year has gone by, and we look forward to an even more eventful and challenging year ahead. I am sure everyone in the PSI family is enjoying their own research work and excited about the new developments in the field. Journal of Proteins and Proteomics, administered by PSI will continue to serve the scientific fraternity to publish and read new findings in the area of protein science.

Before I close, I would like to encourage more and more participation of young members to keep the spirit high of the Society and request all to make maximum use of the proteomic science in answering research questions, collaborate and continue as an active team. New vision and aspiration from all are most welcome. Let us know how PSI-EC can be of any help to its members.

Looking forward to such cooperation in future and working together to understand the human, animal, plant, micro-organisms and environmental biology with the help of the great proteome science.

Finally, this is an unprecedented and challenging time that all of us are going through. I wish a safe and healthy time ahead and an enjoyable reading of the newsletter to all.

With best wishes for a successful 2020,

Dr. Subhra Chakraborty President, PSI

11th Annual meeting of PSI 2019 and International conference on System Integrated Bio-Omics, One Health and Food Safety" (ICPBHF-2019)

The 11th Annual Meeting of Proteomics Society, India 2019 was held at ICAR-National Dairy Research Institute, Karnal for one week from 28th November 2019 to 4th December 2019. This annual meeting of PSI was organized by organizing 3 different events: "Pre-Conference International Workshop (28th Nov - 1st Dec 2019)", "PSI Education day (1st Dec 2019)" and "International conference on System Integrated Bio-Omics, One Health and Food Safety" (ICPBHF-2019). The ICPBHF-2019 aimed at bringing together renowned national and international researchers working in the areas of proteomics, metabolomics and cell biology under one umbrella to exchange scientific ideas and foster future collaborations. This workshop and conference hosted a mixed scientific crowd of 450 young, intermediate and experienced researchers (faculties from different Universities, medical colleges and research institutes, post-doctoral researchers and PhD scholars) from India and abroad including a galaxy of more than 50 personnel from corporates. The event was organized by 3 different Pre-Workshops, Education day followed with International Conference. The themes of this conference include Omics Technologies for One health and Productivity, Clinical and Disease proteomics in human, animal and plant, Proteomics, PTMs and Big data analysis, Proteogenomics, Metabolomics, Integrative omics and systems biology, Biomarker discovery, Structural proteomics, Drug targets, and entrepreneurship, Proteomics and Mass Spectrometry applications in Food safety and Allergy, and Translational Omics and Entrepreneurship.

Pre-conference-International Workshop on Advanced Proteomics (28th Nov-1st Dec 2019)

The workshop was held during 28th Nov - 1st Dec 2019, at ICAR-NDRI, Karnal, India. The preconference workshop was divided into three independent themes; Targeted Proteomics, Quantitative Proteomics and Proteogenomics. The pre-conference workshop was conceptualized and jointly coordinated by Dr. A. K. Mohanty, Principal Scientist, ICAR-NDRI & Convener, 11th PSI meeting, and Dr. Sanjeeva Srivastava, Professor, IIT, Bombay. The Pre-conference workshop started with an inauguration function which was inaugurated by Dr. A.K. Tyagi, Joint Director, ICAR-NDRI, Karnal. The workshop on Targeted Proteomics was held on 28th Nov, and conducted by Prof. Sanjeeva Srivastava, Professor, IIT Bombay. Opening lecture was delivered by Prof Michael MacCoss for the basics and importance of the skyline tool, which is extensively used for the targeted proteomics followed by Prof. Sanjeeva on the principle of Mass Spectrometry. Altogether, they discussed on the approaches for quantifying the cellular proteome and addressed the ways to incorporate proteome data towards improving present day methodologies for biomarker evaluation and precision medicine. Mr. Saicharan Ghatasala, IIT Bombay, provided the basics of Selected Reaction Monitoring (SRM) & Parallel Reaction Monitoring (PRM) leading with Skyline Demo and SRM Data Analysis. In the afternoon, Ms. Vagisha Sharma, MacCross Lab, Washington University, Seattle introduced the participants with Panorama software used for the submission of the skyline analyzed data. Mr. Vipin Kumar, IIT Bombay, described and provided the demo for the PRM Data Analysis Using Skyline.

On the 2nd day on 29th Nov, the Quantitative Proteomics was jointly coordinated by Dr. Aswini Panigrahi, Director, Proteomics Core Laboratory Washington D.C, USA and Dr. A. K. Mohanty, ICAR-NDRI, Karnal. This workshop focused on how to implement proteomics technologies to answer specific questions about a biological system. It provided an overview of LC-MS/MS data collection, label-based and label-free approaches for relative protein quantitation, methods to study Protein-Protein Interactions; and analysis and interpretation of mass spectrometry data. The workshop started with classes by Dr. Aswini Panigarhi and Dr. A. K. Mohanty. After the classes there was In-lab

demonstration of mass spectrometry platform by Vipin Kumar, IIT, Bombay; sample preparation was demonstrated by Dr Preeti Rawat, ICAR-NDRI and LC-MS/MS data collection was demonstrated. Demonstration of Proteome Discoverer software for database search and compilation was discussed by Dr. Krishna Mane, Thermo Fisher Sci. Hands-on session on analysis of Mass Spectrometry data using Max-Quant and other open-source tools were demonstrated by Dr. Aswini Panigarhi and Mr Syed Azmal Ali, ICAR-NDRI.



On the 3rd and 4th day of the workshop (30th Nov – 1st Nov 2019), Dr. D. R. Mani, Broad Institute, USA and Prof. David Fenyo, New York University, USA, delivered lectures and hands on session on the basics as well as advanced Proteogenomics to make participants aware of the importance of multi-omics analysis. They presented the exclusive statistical and machine learning approaches for analyzing the large proteomic data sets. The main focus was given on Data normalization, missing values and batch correction; Hypothesis testing and adjustment for multiple-testing; Unsupervised and supervised machine learning algorithms; Correlation and outlier analysis; Pathway enrichment analysis; and Cloud computing for implementing many of these algorithms.

The Pre-Conference workshop was a novel concept which was introduced in time with novelty to make the students and academicians aware of the advancement in proteomics which will arouse interest in them for the International Conference. The workshop was attended by more than 100 participants.

PSI-Education Day on Metabolomics tools for addressing biological problems (1st Dec 2019)



The Education Day was organized on 1st Dec. 2020 on Metabolomics combined with the parallel hands on session on Terra and R code by Dr. Prasad Phapale, EMBL, Germany and Dr. Vineeta Rai, University of North Carolina. Dr. Prasad Phapale, EMBL, Germany delivered lectures on LC-MS based Metabolomics workflows. Ms. Vineeta Rai, delivered lecture on the basics of Metabolomics to Metabolism in the context of system biology. Yashwant Kumar, THSTI, Faridabad delivered lecture on experiment design for large-scale metabolomics experiments. Mr. Saurabh Nagpal, Application Scientist, Mass Spec Division, Agilent spoke on comprehensive LC/MS-based tools for discovery and targeted metabolomics: Understanding mechanisms and biology of disease; and Integrating Metabolomics and Proteomics for Comprehensive systems biology approach: MPP. Ms. Shubra Agarwal, Agilent, delivered lecture on Data analysis. Dr. Deepak SA, Product Specialist, Cell Analysis, presented a talk on Gain a new perspective with rapid measurement of metabolic functions in live cells using Seahorse XF Analyzers; and also discussed on functionality of SeaHorse.

11th Annual meeting of PSI 2019 and International conference on "System Integrated Bio-Omics, One Health and Food Safety" (ICPBHF-2019)" 2nd-4th Dec 2019



11th Annual meeting of Conference on Proteomics for System Integrated Bio-Omics, One Health and Food Safety was conducted from 2nd - 4th Dec. 2019 at the ICAR-NDRI, Karnal. On the first day, the conference was inaugurated by Dr. Trilochan Mohapatra, Secretary, DARE & DG, ICAR, who was the chief guest. Dr. Steve Pennington, President, Human Proteome Organization (HUPO), Prof. University College Dublin, Ireland was the guest of honor in the inaugural function. After completion of the inaugural function, the scientific session started in which several eminent scientists from all over the world shared their research work. The first day of the conference was presented by a galaxy of 13 National and International experts. The scientific talks started with presentation of two plenary lectures by Dr. Marc Wilkins, University of New South Wales, Australia and Dr. Sean O' Donoghue, Garvan Institute of Medical Research, Sydney, Australia. The session was chaired by Prof. Mark S. Baker, Macquarie University, Australia. Dr. Wilkins presented his talk on the title "The missing link(s) in proteomics. Network discovery by crosslinking mass spectrometry" and Dr. Sean O' Donoghue spoke on "Visually exploring the 'dark' proteome of structural biology". After completion of the plenary lectures by these eminent speakers, the 1st scientific session started on the theme "Proteomics in One Health" which was chaired by Prof. Stephen Pennington, University College Dublin, Ireland and Prof. Utpal S Tatu, Indian Institute of Science, Bangalore, India. This session aimed at discussing **PSI NEWS LETTER IULY-2020** 08

on the development of proteomics in One health which has immense social impact in the changing environment of interdependent world. The speakers in this session included Prof. David Eckersall, University of Glasgow, UK (Title: Proteomics in veterinary research and one health: current progress and future potential); Prof. Alessio Soggiu, University of Milan, Italy (Title: Unraveling one health microbiomes by a targeted metaproteomics approaches); Suresh Mathivanan, La Trobe University, Australia (Title: Dietary extracellular vesicles in health and disease); Prof. Susana Chistobal, Linköping University, Sweden (Title: Environmental pollution in the One health concept, proteomicsbased solutions).



The presentations in the area of One Health aroused great interest among the participant's and several other renowned scientists. The 2nd scientific session of the conference was on the theme area "Proteogenomics, Integrative omics and System Biology. In this session Prof. Akhilesh Pandey, Mayo Clinic, USA and Dr. Debashis Das, IGIB, India chaired the session. Six key speakers presented their talk on various areas: Dr. D. R. Mani, Broad Institute of MIT and Harvard, USA (Title: "Proteogenomic data analysis methods for biological insight"); Prof. Michael Gillette, Broad Institute of MIT and Harvard, USA (Title: Proteogenomic Landscape of Lung Adenocarcinoma); Prof. David Fenyo, NYU Langone Medical Center, USA (Title: A Proteogenomic Study of Retrotransposition in Cancer); Dr. Harsha Gowda, QIMR Berghofer Medical Research Institute, Australia (Title: Proteogenomic landscape of esophageal squamous cell carcinoma); Dr. Kesav Prasad, Yenepoya University, India (Title: Deep proteomic analysis of sub-anatomic regions of the human brain provides molecular insights into neurological disorder) and Dr. Pratik Jagtap, University of Minnesota, USA (Title: Proteogenomics tools and workflows within Galaxy platform). In the evening, plenary lecture was presented by Prof. Akhilesh Pandey who spoke on the topic "Investigating the Glycoproteome in Health and Disease". Following the plenary session, Poster Session-I was conducted and many participants including students, scholars and young scientists participated actively. The posters were

judged by eminent scientists. The day one majorly focused on the high-throughput integrative Multiomics approaches and the current technologies required for solving the current problems in diverse areas ranging from diseases, stress tolerance, infertility and low yields in animal species including humans. In the evening executive council meeting of PSI was held. The day culminated in poster sessions followed by gala dinner.



The day two was dedicated to Molecular and Cellular Proteomics which started with a plenary talk by Jochem M Schwenk, KTH- Royal Institute of Technology, Sweden who discussed the insights from profiling human plasma proteomes for precision phenotyping. The plenary session was chaired by Dr. Anthony W. Purcell from the Monash Biomedicine Discovery Institute, Australia. Following the plenary session, scientific Session-IV "Cellular and Molecular Proteomics" and "Integrative Bio-omics in Production Systems, Foodomics and Biopharma" started as two parallel the themes. ; Session IV on the theme area Cellular and Molecular proteomics was chaired by Dr. Aleksandra Nita-Lazar, NIH, USA. Seven speakers presented their talks on various research areas on the theme: Dr. Subhra Chakravarty, NIPGR, India (Title: System level understanding of organeller control of chitosan triggered immunity); Dr. Niranjan Chakraborty, NIPGR, India (Title: Stress-induced alterations in the membrane proteome: new insights into the molecular basis of stress adaptation in plants); Shantanu Sengupta, Institute of Genomics and Integrative Biology, India (Title: Vitamin B12 deficiency and the great Indian lipid paradox) Atul Shahaji Deshmukh, University of Copenhagen, Denmark (Title: Mass spectrometry-based proteomics to study skeletal muscle metabolism); Swasti Raychaudhuri, Centre for Cellular and Molecular Biology, India (Title: To aggregate? Or not to aggregate? Conformational switch of RCC subunits to fight against proteostasis stress - a complexome profiling approach); Pradip Behare, National Dairy Research Institute, Karnal (Title: Stress responsive proteotranscriptomics changes in indigenous probiotics lactobacilli) and S. Nishad, Bhabha Atomic Research Centre, India (Title: iTRAQ-based proteomic analysis of human cells exposed to low dose chronic natural background radiation). The parallel Session on "Integrative Bio-omics in Production Systems, Foodomics and Biopharma" was chaired by Prof. Davis Eckersall, University of Galsgow,

UK. Eight speakers presented their talks on various topics on the theme areas. Dr. Tushar K. Maiti, Regional Centre for Biotechnology, India (Title: Proteomics studies on human pregnancy and pregnancy related complication like Preterm Birth in GARBH-Ini Cohort); Dr. T. K. Datta, National Dairy Research institute, India (Title: Looking at the spermatozoa: Beyond motility); Suneel Onteru, (National Dairy Research institute, India Title: Integrated proteo-transcriptome analysis in metabolic tissue during early lactation in bovine); Srinivas Kiran Ambatipudi, Indian Institute of Technology Roorkee, India (Title: Dynamic Bovine Milk Alterations in Health and Mastitis); Manmohan Parida, Defense Food Research Laboratory, India (Title: Foodomics in Food Safety: Proteomic insight for Food Authenticity); Alka Rao, CSIR-IMTECH, India (Title: Antimicrobial peptides for Food Safety); Shunmugiah Karutha Pandian, Alagappa University, India (Title: Explication of Anti-infective potential of bioactives against virulence system of bacterial and fungal pathogens through proteomics approaches) and Mukunda Goswami, Central Institute of Fisheries Education, India (Title: Fish Proteomics Research: National and International Perspectives).



Scientific session III on Quantitative Proteomics, which could not be held on the first day was scheduled as a parallel session with Scientific session V on PTM Proteomics. Session on Quantitative proteomics was chaired by Dr. Aswini Panigrahi, Children's National Medical Center, Washington, USA and Krishnan Venkataraman, Vellore Institute of Technology, India. The key speakers in this session were Dr. Anthony A. High, St. Jude Children's Research Hospital, USA (Title: Quantitative Proteomics Initiatives in Basic Science and Cancer Therapeutics); Srikanth Rapole, National Centre for Cell Science, India (Title: Proteomics and functional study reveal MZB1 and XPO1 as potential targets for Multiple myeloma); Amit Kumar Yadav, Translational Health Science and Technology Institute, India (Title: Multiplexed quantitative proteomics data analysis and visualization- the next frontier is now); Suruchi Aggarwal, Translational Health Science and Technology Institute, India (Title: QuantWizIQ- A tool for protein quantitation based on isobaric tags from tandem mass spectrometry) and Rajiv Kumar, Institute of Himalayan Bioresource Technology, India (Title: Metabolic signatures provide novel insights to Picrorhiza kurroa adaptation along the altitude in Himalayan region).

Session V on PTM Proteomics was chaired by Dr. Abhijit Chakrabarti, Saha Institute of Nuclear Physics, India and Prof. Susana Chistobal, Linköping University, Sweden. The session started with a plenary lecture by Prof. Chuna Ram Choudhary, University of Copenhagen, Denmark (Title: Proteomewide analysis of lysine acetylation); Other lead speakers were Dr. Aleksandra Nita-Lazar, National Institutes of Health, USA (Title: ADP-ribosylation regulates the innate immune signaling in macrophages); Dr. Mahesh Kulkarni, National Chemical Laboratory, India (Title: Glycation of glucose sensitive lysine residues K36, K438 and K549 of albumin is associated with prediabetes); Dr. Tryambakam Basak, Indian institute of Technology Mandi, India (Title: Deciphering site-specific collagen post-translational modifications using high resolution Mass Spectrometry); Prof. Krishnaswami Balamurugan, Alagappa University, Karaikudi, India (Title: Analysis of post translational modifications in Caenorhabditis elegans against bacterial exposures). Session V was followed by Poster Session-II post Lunch. Session V on the theme area "Clinical and Disease Proteomics" which was chaired by Dr. Surekha M. Zingde, formerly from Cancer Research Institute, Tata Memorial Centre, Mumbai and Dr. Arun Badyopdhyay, Indian Institute of Chemical Biology, India. In this session, there were 8 lead presentations by eminent speakers. Various speakers who presented in this session were Prof. Jeroen Krijgsveld, German Cancer Research Center, Germany (Title: Novel workflows for quantity-limited clinical proteomics); Prof. Utpal S. Tatu, Indian Institute of Science, India (Title: Proteomic characterization of a mega genome harboring protozoan Infectious agent of trichomoniasis); Dr. K. Dharmalingam, Aravind Medical Research Foundation, India (Title: Proteomics of Host and Pathogen Response in human mycotic keratitis); Prof. Sanjeeva Srivastava, Indian Institute of Technology Bombay, India (Title: Quantitative and functional proteomics based investigations of brain tumors); Dr. Ramesh Ummanni, Indian Institute of Chemical Technology, India (Title: Tumor protein D52 (TPD52) an understudied oncogene; a promising target for prostate cancer) and Dr. A R Suryawanshi, Institute of Life Science, India (Title: Proteomics Multi-approach leads to Identify Differentially Expressed Plasma Proteins Involved in Nasopharyngeal Carcinoma.; Deepa Bisht, National JALMA institute of Leprosy, India Title: Proteome profiling of Mycobacterium tuberculosis isolates resistant to amikacin and kanamycin. The plenary session was scheduled in the evening of 2nd day after completion of session VI. Dr. Aleksandra Nita-Lazar, NIH, USA chaired this session. The plenary talks were presented by two eminent scientists. Prof. Mark S. Baker, Macquarie University, Australia presented his talk on "Colorectal cancer SWATH studies (human health) and collective human proteome project" and Prof. Henning Hermjakob, EMBL-EBI, UK presented his talk on "Pathway-based Omics Data Analysis and Integration". PSI general body meeting was held after the plenary lectures.

The day culminated with a cultural program Jashne-e-Lok which was organised by ICAR-NDRI featuring traditional Haryanavi and Punjabi dance and rhythm segments.

The last day of the conference focused on Biomarker Discovery, Translational Omics and Entrepreneurship. The plenary talk was presented by Prof. Stephen Pennington, University College Dublin, Ireland on the topic "Development and Delivery of Advanced Protein Biomarker Tests for an Era of Precision Medicine". This session was chaired by Prof. Jeroen Krijgsveld, German Cancer Research Center, Germany. After completion of the plenary lecture, scientific session VII on the theme "Biomarker discovery, Translational Omics and Entrepreneurship" was held. This session was chaired by Prof. Henning Hermjakob, EMBL-EBI, UK and Dr. Alessio Soggiu, University of Milan, Italy. Eight eminent speakers spoke on various topics on the theme. The talks were highly interesting and interactive: Prof. Anthony W. Purcell, Monash Biomedicine Discovery Institute, Australia (Title: Immuno-peptidomics and the Antigenic Landscape of Melanoma); Dr. Aswini Panigrahi, Children's National Research Institute, USA (Title: Discovery and Validation of Pharmacodynamic Biomarkers of



Response to Corticosteroids and Infliximab in Pediatric IBD); Dr. Hariprasad G, All India Institutes of Medical Sciences, India (Title: Protein biomarkers to monitor pharmaco-therapy in dopamine dictated states of Parkinson's disease and schizophrenia); Dr. Sudarshan Kumar, National Dairy Research Institute, India (Title: TMT label based quantitative proteomics discovers biomarker for the detection of subclinical mastitis in cows); Dr. M. Vijaylakshmi, IITM, India (Title: Translational Research and Entrepreneurship); Prof. Ashish Mukherjee, Tezpur University, India (Title: Proteomics of Indian Monocled cobra of North East India and its cross-reactivity by antivenomics); Dr. Kaushik Dey, St. Jude Children's Research Hospital, USA (Title: Mass Spectrometry-based biofluid Proteomics toward Biomarker discovery for Alzheimer's disease) and Dr. Renu Goel, Translational Health Science and Technology Institute (THSTI), India (Title: Plasma proteomics approach to understand the temporal expression of proteins after live donor hepatectomy at different stages of liver regeneration). by The final session VIII of the conference was on the theme area "Metabolomics in Biological applications". Six speakers presented their talk on various research areas. The talks presented were by Prof. Pavneesh Madan, University of Guelph, Canada (Title: Use of Omics & Machine Learning Tools for Predicting Embryonic Health & Viability); Dr. Prasad Phapale, EMBL, Germany (Title: 13C Stable Isotope tracer Metabolomics to study spatial metabolism); Dr. Ranjan Nanda, International Centre for Genetic Engineering and Biotechnology (Title: Deregulated amino acid metabolism and dysbiosis in tuberculosis); Dr. Sanjib Meitei, PREMIER Biosoft, USA (Title: Informatics Support for Untargeted and Targeted Methods in Mass Spectrometry-Based Lipidomics); Dr. Vineeta Rai, North Carolina State University, USA (Title: Iron limitation induces root exudate metabolites, shapes tomato rhizosphere microbiota) and Dr. A K Balhara, Central Institute for Research on Buffaloes, India (Topic: Buffalo urinary metabotyping during early pregnancy) This conference acted as a global platform where scientists from diverse areas across the globe share and discuss ideas to solve the grassroots problems relating to health and diseases in animals and humans.

After the completion of scientific sessions, the valedictory function was held. The awards were given for the best oral and poster presentations, travel awards. The valedictory function was chaired by Dr. RRB Singh, Director, ICAR-NDRI, Karnal. Prof. M. L. Madan, Former Vice-Chancellor of Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, India and Prof. Susana Cristobal, Linkoping University, Sweden were invited as distinguished guests in the valedictory function. Dr. Ashok K. Mohanty, Organizing Secretary briefed the audience about the overall arrangement of the conference and how the entire event was organized and thanked one and all for their active support to organize the event. Dr. RRB Singh expressed his deep sense of gratitude to all the distinguished guests for visiting ICAR-NDRI, Karnal and making the 11th Annual



Annual Meeting of PSI a great success. On the behalf of organizing committee Dr Sudarshan Kumar, SS, ABTC, NDRI, thanked all the eminent speakers, session chairs, participants, and sponsors for making the PSI-2019 successful. The venue for 12th Annual PSI meeting was proposed to be held at CCMB, Hyderabad in the year 2020.

HUPO 2019

18th Human Proteome Organization World Congress was held in Adelaide, Australia during 14th to 19th September, 2019. The Congress was hosted by the Australasian Proteomics Society (APS). The congress provided a world class scientific program with outstanding international speakers at the very forefront of proteomics and associated fields, alongside with an exceptional spotlight for students and early career researchers.

Proteomic Society, India, supported five students with Travel Awards to attend the meeting. The research work presented by them at this meeting is given in the next few pages.

<u>Chemical modification of proteins to mimic LysC proteolysis: Application of 1,2-</u> <u>dicarbonyl compounds for arginine modification</u>

Boomathi P Pandi1¹, Nagarjuna R Chary², A.S. Kamalanathan1, Sripadi Prabhakar², Varatharajan Sabareesh¹

1. Advanced Centre for Bio Separation Technology (CBST), Vellore Institute of Technology (VIT), Vellore - 632014, Tamil Nadu, India

2. Centre for Mass Spectrometry, Analytical Department, CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, Telangana - 500007, India



Introduction: We are interested to develop a new method for an emerging area, middle-down proteomics (MDP). In MD approach, proteases such as LysC, AspN, etc. are used to produce longer proteolytic peptides, which yields better sequence coverage than bottom-up approach and hence, post-translational modifications can be detected more reliably (e.g., in histones and antibodies). We apply the strategy of modifying the guanidine side chain of arginines of proteins by 1,2-cyclohexanedione (CHD) or phenylglyoxal (PG), prior to trypsin digestion, which would result in

'longer tryptic peptides' and such arginine-modified tryptic peptides mimic LysC derived peptides.

Methods: Before applying this method for proteomics, we investigated five model proteins: β -lactoglobulin, β -casein, RNase A, ovalbumin and human transferrin. Carbamidomethylation of proteins was done before arginine modification and ~100 molar excess of CHD or PG was used (16 hrs, pH 8.4 (borate), ~250 C) for arginine modification reactions. Subsequently, each sample was digested with trypsin (370C) for different incubation times, which was monitored by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS: 1290 Infinity LC attached to 6540 or 6545 Q-TOF (Agilent) and Acquity UPLC attached with Quattro Premier XE (Waters).

Findings: Three arginine-modified tryptic peptides of lengths in the range: ~26 - 50 amino acid residues (a.a.r), were detected from each of β -lactoglobulin and β -casein. In all these modified tryptic peptides, one molecule of CHD or PG was covalently added to the sidechain of the arginine residue. Tryptic peptides of very short lengths (< 5 a.a.r) and not longer than 25 a.a.r. were not observed from arginine-modified RNase A. Similarly, longer arginine-modified tryptic peptides were observed in other model proteins as well.

Conclusion: Thus, in cases, where LysC is useful for MD approach based proteomics and protein sequencing, our strategy of arginine-modification-cum-trypsin digestion can be a new approach, which can be an alternative method and cost-effective too.

References:

1. Takahashi, K., (1968). The Reaction of phenylglyoxal with arginine residues in proteins. J. Biol. Chem. 243, 6171-6179

2. Patthy, L., and Smith, E.L. (1975). Reversible modification of arginine residues. J. Biol. Chem. 250, 557-564

3. Brock, J.W.C., Cotham, W.E., Thorpe, S.R., Baynes, J.W., and Ames, J.M. (2007). Detection and Identification of arginine modifications on methylglyoxal-modified ribonuclease by mass spectrometric analysis. J.Mass Spectrom. 42, 89-100

<u>Autoantibody Response Against Tumor-Associated Antigens in Gallbladder</u> <u>Carcinoma Using Immunoproteomics approach</u>

^{1,2}Javed Akhtar, ^{1,2}Ratna Priya, ¹Vaishali Jain, ³Puja Sakhuja, ³Anil Kumar Agarwal, ⁴Ravindra Varma Polisetty, ⁵Ravi Sirdeshmukh, ²Sudeshna Kar, ¹Poonam Gautam

¹ICMR- National Institute of Pathology, Safdarjung Hospital Campus, New Delhi-110029, India; ²Jamia Hamdard-Institute of Molecular Medicine, Jamia Hamdard (Hamdard University), New Delhi-110062, India; ³Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi- 110002, India; ⁴Department of Biochemistry, Sri Venkateswara College, University of Delhi, New Delhi-110021, India; ⁵Institute of Bioinformatics, International Tech Park, Bangalore-560066, India.



Background: Early diagnosis is important for timely treatment of gallbladder carcinoma (GBC) patients leading to the increased survival rate. Here, we have applied serological proteome analysis (SERPA), an immunoproteomics approach, for detection of tumor-associated antigens (TAAs) eliciting humoral response in early stage GBC patients.

Methodology: Immunodepleted tissue proteins from GBC patients (n=7) were resolved by two - dimensional gel electrophoresis (2-DE) followed by

immunoblotting using pooled blood plasma from healthy volunteers (n=11) or gallstone (GS) cases (n=11) or early stages of GBC (n=5) or advanced stages of GBC cases (n=9). Image analysis was performed using PDQuest software to identify protein spots with significantly high or specific immunoreactivity in GBC cases. The corresponding protein spots were excised from the 2-D gel followed by in-gel trypsin digestion and mass spectrometric analysis (LC-MS/MS) for identification of proteins. Two of the identified proteins were verified for autoantibody levels in individual plasma samples (30 cases and 20 controls) by dot blot assay.

Findings: 2-D immunoblot analysis led to identification of 25 protein spots showing either significantly high or specific immunoreactivity in early and/or advanced stages of GBC. Mass spectrometric analysis led to identification of proteins from the immunoreactive spots, including annexin A1 (ANXA1), and heat shock protein 60 (HSP60), carbonic anhydrase isoform 1 and 2, aldolase B and cathepsin D. Evaluation of autoantibody levels in individual plasma samples against two of the recombinant proteins, ANXA1 (an immunomodulatory protein implicated in cancer) and HSP60 (chaperonin involved in regulating apoptosis in cancer) using Dot blot assay showed significantly higher levels of autoantibodies against HSP60 (unpaired t-test, p= 0.023) in early stage GBC cases.

Conclusions: The study suggested that the autoantibody levels against HSP60 may be potentially employed for detection of GBC patients at early stages, however, the autoantibody level needs to be validated in larger cohort of clinical samples.

Keywords: Autoantibody; Gallbladder carcinoma; Immunoblotting

<u>Dehydration-responsive nuclear proteome and phosphoproteome profiling of a</u> grain legume chickpea (*Cicer arietinum* L.)

Pragya_Barua, Niranjan Chakraborty

National Institute of Plant Genome Research, New Delhi, DELHI, India



Background: Human health is deeply rooted with the natural resources, which provide nutrition, and many current research emphasize in underpinning sustainable planetary health. With the socio-economic impacts of climatic drifts on the rise, the yield of nitrogen fixing legumes is being adversely affected by dehydration stress. Identifying the organelle-specific regulators for adapting to such environmental constraint would not only aid in understanding the molecular basis of stress-response, but developing fortified varieties (1). Nucleus (PM), designated as the cell's control centre, hosts genetic information and regulates gene

expression, we therefore aimed at understanding the dehydration-induced alterations in the expression patterns of the proteins and phosphoproteins hosted by the nucleus.

Methodologies: Four-week-old seedlings of a grain legume, chickpea, were subjected to gradual dehydration (2) and nuclear proteins (NPs) were extracted from unstressed control as well as from 72 and 144 h stressed tissues. Phosphopeptides were enriched by titanium dioxide treatment followed by detection and relative quantification.

Findings: We identified 4832 NPs and 478 phosphosites, corresponding to 299 unique nuclear phosphoproteins (NPPs) involved in multivariate cellular processes including protein modification and regulation of gene expression, among others (3). The identified proteins included several novel kinases, phosphatases and transcription factors, besides 660 uncharacterised proteins. Spliceosome complex and splicing related proteins were dominant among differentially regulated NPPs, indicating their dehydration-modulated regulation. Phospho-motif analysis revealed stress-induced enrichment of proline-directed serine phosphorylation. Association mapping of NPPs revealed predominance of differential phosphorylation of spliceosome and splicing associated proteins. Also, regulatory proteins of key processes viz., protein degradation, regulation of flowering time and circadian clock were observed to undergo dehydration-induced dephosphorylation.

Concluding statement: This inventory comprising several novel regulatory proteins and their precise sites of phosphorylation, would provide new insights into stress adaptation and enable directed genetic manipulations for developing climate-resilient crops.

References:

1. Gayen, D., Barua, P., Lande, N. V., Varshney, S., Sengupta, S., Chakraborty S, Chakraborty, N. (2019). Dehydrationresponsive alterations in the chloroplast proteome and cell metabolomic profile of rice reveals key stress adaptation responses. Environ Exp Bot 160: 12-24.

2. Gayen, D., Gayali, S., Barua, P., Lande, N. V., Varshney, S., Sengupta, S., ... & Chakraborty, N. (2019). Dehydrationinduced proteomic landscape of mitochondria in chickpea reveals large-scale coordination of key biological processes. J Proteomics, 192: 267-279.

3. Barua P, Lande NV, Subba P, Gayen D, Pinto S, Keshav TS, Chakraborty S, Chakraborty N. (2019) Dehydration responsive nuclear proteome landscape of chickpea (Cicer arietinum L.) reveals phosphorylation-mediated regulation of stress response. Plant Cell Environ 42: 230-244.

17

Role of NCL1 in Cysteine induced toxicity

Rahul Chakraborty, Ajay Bhat, Khushboo Adlakha, Monu kumar Chadar¹, Kausik Chakraborty^{* 1,2} and Shantanu Sengupta^{*1,2}

¹CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi, India ²Academy of Scientific and Innovative Research, New Delhi, India



Cysteine, a thiol containing amino acid synthesized via the transsulfuration pathway is a rate limiting precursor for glutathione synthesis and is also utilized for protein synthesis. Among the biological thiols elevated levels of homocysteine is known to be associated with various diseases and is considered to be an independent risk factor for cardiovascular disease. Recent evidences actually support this fact and it has been shown that cysteine induced growth defect in yeast was more severe than homocysteine. Reports also suggest that elevated levels of cysteine may be associated with cardiovascular disease. Using Yeast as a model

system in this study we have tried to figure out the mechanism of cysteine induced toxicity. To characterize the cellular response in presence of high level of cysteine we have performed a quantitative proteomics experiment and found several differentially expressed proteins in presence of high level of cysteine which includes aminoacid metabolic proteins, glycolytic – TCA cycle proteins, ribosomal proteins. Even after genomewide mutant screening we came to know that Ncl1 (SAM dependent t-RNA methyl transferase) plays a crucial role in Cysteine toxicity. Δncl1 is much more sensitive towards cysteine and it plays a crucial role in protein biosynthesis and energy metabolism.Intracellular aminoacid measurement by using o-phtalaldehyde reveals that cysteine causes aminoacid imbalance in the cells and by using s35 labeled methionine we also found that cysteine induces translational arrest. Further we have found that supplementation of high levels of leucine and pyruvate can rescue cysteine induced toxicity.

<u>Multi-omics analyses reveal temporally distinct metabolic switches, carbon</u> <u>nitrogen partitioning and oxidative signaling in chickpea seed</u>

Arunima Sinha, Toshiba Haider, Kanika Narula, Sudip Ghosh, Niranjan Chakraborty, Subhra Chakraborty

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi-110067, India.



Background: Nutrient dynamics in storage organs is a complex developmental process that requires coordinated interactions of environmental, biochemical, and genetic factors. It correlates with metabolically programmed progressive differentiation of genetically distinct compartments in seed. Nutrient signals and metabolic adaptations determine differentiation pattern and transition from maternally-controlled embryonic growth to maturation under filial regulation. Although sink organ developmental events have been identified, our understanding of transcriptional, translational, post-translational and metabolic

regulation of reserve synthesis, accumulation and utilization is limited.

Method: Chickpea seeds were collected at different developmental stages (7-60 DAF) and germination stage. RNA-seq was performed using Illumina Hi-seq 2000 paired-end sequencing technology. Proteome and phosphoproteome were developed using 2-DE and subsequent Pro-Q Diamond staining. Further, TiO2 based phosphopeptide enrichment was done followed by identification of phosphoproteins using TripleTOF mass spectrometer. Integrated global network was built using cytoscaape. Furthermore, qRTPCR analysis was performed to validate the omics datasets.

Results: To understand nutrient dynamics during embryonic and cotyledonary photoheterotrophic transition to mature and germinating autotrophic seeds, an integrated transcriptomics, proteomics and phosphoproteomics study in six sequential seed developmental stages in chickpea was performed. Differential gene expression analysis led to the identification of 6582 nutrient-associated transcripts predominantly involved in primary metabolism in synthesis phase, while downregulation of these pathways characterise the accumulation phase. Resume of central metabolism was observed in nutrient utilization phase. MS/MS analyses identified 175 and 78 nutrient-associated proteins/phosphoproteins (NAPs/NAPPs) related to metabolism, storage and biogenesis, and protein turnover. Identification of sitespecific phosphorylation of amino acids indicated their possible effect in nutrient dynamics. Network analyses identified three significant modules centered around HSP70, vicilin, chalcone synthase and SBP65.

Conclusions: Our study identified several potentially interesting nutrient-associated transcripts and proteoforms of biological significance. Altogether, these findings demonstrate that nutrient signals act as metabolic and differentiation determinant governing storage organ reprogramming.

Keywords: Seed, chickpea, multi-omics, nutrient dynamics

Sinha A, Haider T, Narula K, Ghosh S, Chakraborty N and Chakraborty S (2020) Integrated seed proteome and phosphoproteome analyses reveal interplay of nutrient dynamics, carbon-nitrogen partitioning and oxidative signaling in chickpea. Proteomics 20: 1900267

Serum Small Extracellular Vesicles Proteome of Tuberculosis Patients Demonstrated Deregulated Immune Response

Rakesh Arya^{1,2}, Deepti Dabral^{1,3}, Hossain Md. Faruquee^{1,4}, Himanghsu Mazumdar⁵, Saurav Jyoti Patgiri^{5,6}, Trinayan Deka⁷, Rumi Basumatary⁷, Rukuwe-u Kupa⁸, Chayale Semy⁸, Wetetsho Kapfo⁸, Kevideme Liegise⁸, Inderjeet Kaur⁹, Tenzin Choedon¹⁰, Purnima Kumar¹¹, Rajendra Kumar Behera², Pranjal Deori¹², Reema Nath⁷, K. Pewezo Khalo¹³, Lahari Saikia^{5,6,14*}, Vinotsole Khamo^{8*}, Ranjan Kumar Nanda^{1*}.

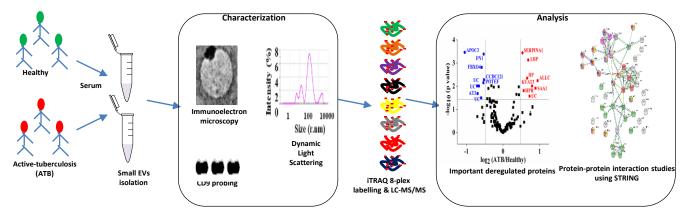
¹Translational Health Group, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India. ²School of Life Sciences, Sambalpur University, Sambalpur, Odisha-768019, India. ³Molecular Physiology, and Molecular Medicine Research Group, School of Medicine, Western Sydney University, Campbell town Campus, NSW-2560, Australia.⁴Department of Biotechnology & Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh. ⁵Multidisciplinary Research Unit (ICMR), Assam Medical College, Dibrugarh, Assam-786002, India. ⁶Regional Medical Research Centre, N.E. Region (ICMR), Post Box No.105, Dibrugarh, Assam-786 001, India. ⁷Department of Microbiology, Assam Medical College, Dibrugarh, Assam-786002, India. ⁸Healthcare Laboratory and Research Centre, Nagaland Hospital Authority Kohima, Nagaland-797001, India. ⁹Malaria Biology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India. ¹⁰Transcriptional Regulation Group, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India. ¹¹Cellular Immunology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India. ¹²Department of TB and Chest Disease, Assam Medical College, Dibrugarh, Assam-786002, India. ¹³Department of Microbiology, Nagaland Hospital Authority Kohima, Nagaland-797001, India.. ¹⁴Department of Microbiology, Guwahati Medical College, Guwahati, Assam-781032, India.

Email- vkhamo@gmail.com, lahari.saikia@yahoo.com, ranjan@icgeb.res.in.

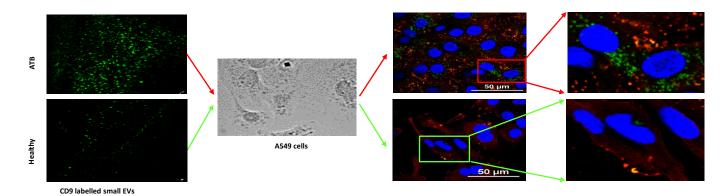
Tuberculosis, a communicable disease caused by Mycobacterium tuberculosis (Mtb) infection usually affects the lungs. Although, effective treatment is available, tuberculosis associated deaths and new cases are increasing due to non-availability of effective diagnostic tests and lack of detailed understanding of patient pathophysiology at case presentation. Understanding disease pathophysiology is key for futuristic solution development for better disease management. Omics analysis provide useful tool to identify bacterial biomolecules such as proteins, peptides, lipids, mRNA etc. or hostderived molecules which get perturbed during infection. Upon infection, Mtb releases its biomolecules such as DNA, RNA, proteins etc. in the granuloma which get transferred to multivesicular endosomes (MVEs), assimilated into intraluminal vesicles and secreted as Extracellular Vesicles (EVs) during MVE fusion with the plasma membrane. Identification of important deregulated host molecules in small EVs or exosomes can explain the altered host pathophysiology observed in tuberculosis patients in greater detail. Following the ethical committee's approved protocol, study subjects were recruited from Assam Medical College, Assam and Nagaland Hospital Authority Kohima, Nagaland. Serum small EVs were isolated using ExoQuick® and ultracentrifugation methods. Isolated small EVs were characterized using immunoelectron microscopy by monitoring surface CD9 expresison and size calculation was carried out using Dynamic Light Scattering. Extracted small EV proteins from active tuberculosis patients (+ve sputum microscopy and nucleic acid amplification test results) and healthy controls were subjected to an isobaric tag for relative and absolute quantification (iTRAQ) experiment

Out of 132 host proteins identified, a set of 17 proteins qualified the criteria of a 1.4 fold change cutoff (\log_2 fold change >±0.48) and p<0.05 were further validated. From STRING and hierarchical clustering analysis, 4 out of 17 important proteins (KYAT3, SERPIAN1, HP and APOC3) were selected for further validation using independent samples by Western blot analysis. Isolated serum small EVs, irrespective of tuberculosis patients or healthy subjects, upon incubation with A549 cells showed time dependent internalization. This confirms that the small EVs isolated from serum of study subjects were still functional and can be exploited for their biological role. In the present study, we showed that proteome composition of serum small EVs isolated from tuberculosis patients are different from that of healthy controls. A set of important proteins that are involved in different biological processes, cellular components and compromise immune response were identified and validated. Taken together, these data suggest that serum small EVs proteome composition analysis may be useful to understand host pathophysiology and develop alternate host-directed therapeutic interventions.

Isolation and characterization of serum small EVs to identify important deregulated proteins in pulmonary tuberculosis:



Functional Analysis:



Reference:

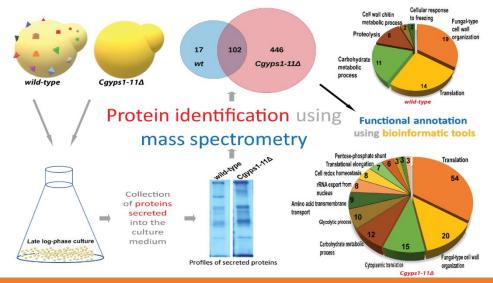
Arya R, Dabral D, Hossain MF, Mazumdar H, Patgiri SJ, Deka T, Basumatary R, Kupa R, Semy C, Kapfo W, Liegise K, Kaur I, Choedon T, Kumar P, Behera RK, Deori P, Nath R, Khalo K, Saikia L, Khamo V, Nanda RK. (2019) Serum Small Extracellular Vesicles Proteome of Tuberculosis Patients Demonstrated Deregulated Immune Response. *PROTEOMICS-Clinical Applications* 18: 1900062.

Global secretome characterization of the pathogenic yeast Candida glabrata

Mubashshir Rasheed¹, Naveen Kumar¹ and Rupinder Kaur^{1*}

¹Laboratory of Fungal Pathogenesis, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, Telangana State, India. (*Email: <u>rkaur@cdfd.org.in</u>)

The incidence of nosocomial bloodstream infections (BSIs), caused by fungal pathogens, is increasing worldwide. Candida spp. are the leading causal agents of fungal BSIs, with Candida glabrata being the second to fourth most prevalent species based on the geographical region. Although C. glabrata infections are associated with a high rate of morbidity and mortality, the virulence traits of this important pathogenic yeast are yet to be delineated in full. A family of eleven putative glycosylphosphatidylinositol (GPI)-anchored aspartyl proteases (CgYapsins) has previously been reported to be essential for the pathogenesis of C. glabrata. Since secretory proteins of pathogens play a critical role in modulating host-pathogen interactions, we, here, have profiled the secretome of C. glabrata through liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach. We showed that the C. glabrata secretome contained 119 proteins primarily involved in cell wall organization, carbohydrate metabolism, proteolysis and translation processes. Importantly, eight CgYapsins including the major proteases, CgYps1 and CgYps7, were found to be part of the C. glabrata secretome. Further, secretome characterization of the Cgyps1-11 Δ mutant (lacks all eleven CgYapsins) revealed 4.6-fold higher number of secretory proteins, compared to the wild-type secretome. Of 548 proteins present in the mutant secretome, only 12% proteins carried the classical secretory signal peptide sequence highlighting the role of the non-conventional secretion pathway in the mutant. Notably, the label-free quantitative secretome analysis identified 65 proteins to be differentially abundant, with 49 and 16 proteins showing increased and decreased abundance, respectively, in the Cgyps1-11∆ mutant secretome. Intriguingly, the abundance of CgMsb2, a putative CgYapsin substrate, was about 85-fold reduced in the Cgyps1-11 Δ secretome. Overall, besides paving the path to a better understanding of the role of secretory proteins in the virulence of C. glabrata, our proteomic and functional analysis demonstrate CgYapsins to be the bona fide constituents and regulators of the secretome for the first time.



Reference:

Rasheed M, Kumar N, and Kaur R. (2020) Global secretome characterization of the pathogenic yeast Candida glabrata. J. Proteome Res. 19 (1): 49-63 [*PMID*: 31621333]

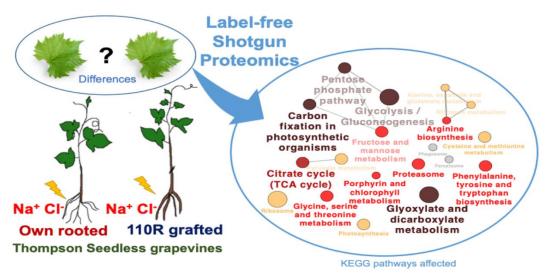
<u>Comparative Proteomics Unravels the Differences in Salt Stress Response</u> of Own Rooted and 110R Grafted Thompson Seedless Grapevines

Sucheta Patil^{1,2}, Manisha Shinde³, Ramya Prashant¹, Narendra Kadoo^{1,2}, Anuradha Upadhyay³, and Vidya S. Gupta^{1,2*}

¹ Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune, India
² Academy of Scientific and Innovative Research, Ghaziabad, India
³ ICAR-National Research Centre for Grapes, Pune (*Email: <u>vs.gupta@ncl.res.in</u>)

Abstract

Thompson Seedless, a popular table-grape variety, is sensitive to salinity when grown on its own roots. Soil salinity severely hampers the plant growth leading to a decrease in the productive lifespan of grapevine. Therefore, farmers have now switched to grafting of grapevines on wild rootstocks for drought and salt stress tolerance. However, the mechanism of salt tolerance through grafting on wild root stocks is not clear and not studied in depth. In the present study, we investigated the differences in response to salt stress between salinity sensitive, own-rooted Thompson Seedless (TSOR), and salinity tolerant, 110R grafted Thompson Seedless (TS110R) grapevines through physiological and molecular studies. The salt stress was imposed by irrigating grapevines with a 150 mM NaCl solution. The salinity induced changes in protein abundance in comparison to those in unstressed vines were quantitated using label-free shotgun proteomics approach across early (6 hours), mid (48 hours), and late (7 days) stages of salt treatment. The abundance of 246 out of 2793 proteins, identified in the study, was significantly affected by salt stress at various time-points in TSOR and TS110R vines which, represented several biological processes such as photosynthesis, amino acid metabolism, translation, chlorophyll biosynthesis and generation of precursor metabolites. The results revealed that the TSOR grapevines displayed diversion of photo assimilates towards osmotic adjustments and tolerance of oxidative-stress while, TS110R grapevines displayed specific upregulation of proteins responsible for prevention of oxidative-stress itself. The findings of this study add to the knowledge of salinity response in woody and grafted plants and hence open the scope for further studies on salt stress-specific differences induced by grafting.



SSABMS 2019 and Metabolomics Workshop at SINP, Kolkata

A school and a symposium focused on Advances in Biomedical Mass Spectrometry (SSABMS 2019) was organized by the Biophysics and Structural Genomics Division of SINP, Kolkata during November 11-14, 2019 followed by a hands-on workshop on Metabolomics during November 15-17, 2019. In addition to invited national and international experts, the school and the symposium was attended by more than hundred participants comprising students, research scholars, clinicians and PIs from all over India. The aim of the school was to introduce the diverse audience to the basic concepts and their applications in biomedical mass spectrometry. The invited lectures in the symposium showcased latest advances in the field and further illustrated the prowess of mass spectrometry (MS) in fundamental research as well as their translation to the clinic.

The school was inaugurated by the Director, Prof. Gautam Bhattacharyya, who welcomed all, touched briefly upon the rich history of the institute as well as the division and wished success to the event. The first tutorial in the school was delivered by Prof. Jennifer Van Eyk (Ceders-Sinai Medical Center, USA) on application of proteomics in precision medicine. With her characteristic flare, she described basics of targeted proteomics and how it is being coupled with recent advances in paperbased remote sampling methods to develop pipeline for longitudinal monitoring of the proteome dynamics, touched upon the challenges in implementation while it eventually leads us towards personalized monitoring of cardiovascular health at population level. This was followed by a tutorial by Prof. Philipp-Britz McKibbin (McMaster University, Canada) on metabolomics. He discussed fundamental principles and considerations for MS-based metabolomics coupled with capillary electrophoresis (CE) in detail and illustrated them using case studies on Phenylketonurea and Cystic Fibrosis. The post-lunch session began with the lecture on principles of MS-based lipidome analysis by Prof. Gavin Reid. Starting with detailed classification of lipids and examples of aberrant lipid biosynthesis in cancer, he went on discuss analysis of different lipid classes as well as challenges that lie in identification of isobaric lipids. He summarized different existing MS-based methods for precise identification of fatty acyl side-chains in complex lipids including examples of Paterno-Buchi reaction and Ozone-induced dissociation. Dr. Ingela Lanekoff (Uppsala University, Sweden), then delivered a lecture on imaging mass spectrometry. Along with the different MS-platforms that are used for imaging of biomolecules such as SIMS, MALDI, LAESI, DESI and principles behind them, she also mentioned recent developments in nano-DESIMS that enabled single-cell imaging. Prof. David Wishart (University of Alberta, Canada), a stalwart in development of bioinformatic tools and databases for metabolomics, delivered the last lecture of the day on MS-based metabolite Identification and annotation using the web. Step-by-step, he covered different approaches for analysis of metabolomic data highlighting the use of freely available web-based tools as well as databases to identify and annotate metabolites and metabolic pathways.

The second day of the school opened with the lecture by Prof. Alex Van Belkum (bioMerieux Corp., France) on MS-based analysis of microbes describing principles behind analysis and characterization of microbes using MALDI-MS, their efficacy and advantage vis-a-vis existing methods that have already made MS-based microbial diagnostics commercially viable. He also touched upon capabilities of MS-based approaches beyond conventional identification, particularly, analysis of anti-biotic susceptibility and elucidation of mechanistic connections. This was followed by

SSABMS 2019 and Metabolomics Workshop at SINP, Kolkata

a lecture by Dr. Daniel Globisch (Uppsala University, Sweden) on investigation of host microbiota cometabolism as a new strategy for biomarker discovery. This fascinating lecture showed how unique chemoselctive probes and biochemical modifications can be combined with the prowess of mass spectrometry to discover novel metabolites towards better understanding of host-microbiome interaction and their implications in disease mechanism and diagnostics. The next lecture by Dr. Pritam Sukul (University of Rostock, Germany) MS-based non-invasive breath analysis elaborated how the volatome is affected by a plethora of factors including physiological as well as physical state of the subject, ambient atmospheric condition as well as mode of collection. It showed that although disease associated changes in breath volatome are not unreal; the path of identification of volatile molecules as biomarkers needs to be cautiously trodden. The concluding lecture of the school by Prof. Eyal Gottlieb (Technion, Israel) eloquently described how stable isotope-based metabolic flux analysis can help to uncover the fundamental mechanism underlying pathogenesis and their connection with genetic factors. He illustrated it with examples of effects of several genes, known to be mutated in cancer, on metabolic flux. In addition to question answer sessions after each lecture, both days concluded with open and informal interaction sessions between speakers and participants where queries regarding fundamental principles, study design, sample preparation to data analysis as well as their implication on our understanding of biochemistry were raised and discussed.



Prof. David Wishart engrossed in discussion with one of the attendees over her poster at the symposium.

The symposium opened with the welcome address by Prof. Abhijit Chakrabarti (SINP, Kolkata) followed by the session on 'Metabolism and Metabolomics' that began with an insightful lecture by Prof. Wishart on *in silico* metabolomics to discover the 'dark matter'- the 'known' and 'unknown' unknown metabolites that are estimated to be 90-98% of the metabolome. Prof. Gottlieb delivered a

SSABMS 2019 and Metabolomics Workshop at SINP, Kolkata

fascinating lecture on unravelling new modules and therapeutic opportunities that exploit aberrant metabolic behaviour of cancer cells. Dr. Srikanth Rapole (NCCS, Pune) talked about the metabolic alterations in invasive ductal carcinoma of the breast; Dr. Ranjan Nanda (ICGEB, New Delhi) talked about the implications of metabolic phenotyping in tuberculosis and Dr. Soumen Kanti Manna (SINP, Kolkata), talked about how metabolomics helped in unravelling mechanism and identifying putative biomarkers in alcoholic liver disease and chronic kidney disease, respectively. The next session on 'Biomarkers and Personalized Medicine' opened with the lecture by Prof. Britz-McKibbin on application of CE-MS-based metabolomics to identify biomarkers and unravel mechanism underlying irritable bowel syndrome followed by the lecture by Prof. Van Eyk on automation and high-content using CE-MS platform towards personalized evaluation of cardiovascular health. Dr. Arun Bandyopadhyay (IICB, Kolkata) talked about the proteomic approaches to understand aberrant cholesterol transport in myocardial infarction, which is leading cause of mortality worldwide. Dr. Tushar Kanti Maiti (RCB, New Delhi) talked about salivary and vaginal fluid proteomic signatures associated with pregnancy and complications, which are widespread in India. Prof. Suman Kundu (Delhi University, New Delhi) talked about a novel approach to identify haemoglobin variants using MS. The final session on 'Lipidomics and Imaging' began with the lecture of Prof. Reid on lipidomic hallmarks of colorectal cancer. Dr. Lanekoff talked about tissue and cell imaging using nano-DESIMS. The final lecture of the day by Dr. Shantanu Sengupta (IGIB, New Delhi) nicely elaborated the evolving understanding of the peculiarities in lipidomic changes associated with cardiovascular disease patients in India.

The second-day of the symposium began with 'Proteins and Proteomics' session where Prof. Shyamalava Mazumdar (TIFR, Mumbai) talked about ionization and fragmentation pattern of proteins and peptides useful for structural analysis. Dr. Swasti Raychaudhuri (CCMB, Hyderabad) talked about the proteomic investigations that revealed changes in respiratory complexes associated with proteostasis stress. Dr. Amit Kumar Mandal (IISER, Kolkata) talked about the use of native mass spectrometry to probe the architecture of glycated and glutathionylated hemoglobins. The next session on 'Microbe and Breath Analysis' began with the lecture of Prof. Van Belkum, who presented latest developments in MS-based diagnostic analysis in infectious diseases at bioMerieux. Dr. Globisch talked about the combined use of chemoselective probes and MS for identification of metabolites and their quantitation at fmol level. Dr. Sukul talked about the relationship between metabolic and physiological changes with exhaled breath volatome. The post-lunch session on 'Screening and Diagnostics' began with the lecture of Dr. Augustin Scalbert (IARC, WHO, France) who apprised the audience on the utility of metabolic profiling on identification of risk factors of cancer. Dr. Usha Dave (MILS International India, Mumbai) talked about MS-based diagnosis of inborn errors of metabolism. Dr. Shibdas Banerjee (IISER, Tirupati) talked about the MSI-based diagnosis and stratification of cancer.

The symposium also comprised of poster sessions during lunch and refreshments on both days. The invited speakers were requested to judge posters. They rated the quality of research and presentation by most of the scholars very highly. Based on their feedbacks, three posters were selected for awards and oral presentations. Ms. Nilanjana Ghosh (IIT, Kharagpur) presented her work on

SSABMS 2019 and Metabolomics Workshop at SINP, Kolkata

metabolomic and immunological profiling of asthma COPD Overlap (ACO), Mr. Ramiz Islam (SINP, Kolkata) presented his work on metabolic reprogramming associated with metformin-induced cytotoxicity in cancer cells and Ms. Debaleena Bhowmik (IICB, Kolkata) presented her work on the development of a web server for microbiome to metabolome prediction. In the valedictory session, Prof. Debashis Mukhopadhyay (SINP, Kolkata) did the honours to thank all participants, invited speakers, distinguished guests, scholars, volunteers and administration of the institute for making the event a success. He also thanked our hospitality partners. Last but not the least; he sincerely thanked the institute and the Department of Atomic Energy for generous support as well as Thermo Fisher Scientific, Sciex, Agilent Technologies, Allied Scientific Products and other sponsors.



The symposium was followed by the metabolomics workshop during November 15-17, 2019 attended by twenty-four participants including research scholars, principal investigators, and clinicians from different parts of the country. In the theoretical sessions Dr. Scalbert discussed principles of study design for biomarker analysis, Dr. Manna discussed principles and practical considerations for metabolite extraction and profiling, Prof. McKibbin discussed pattern recognition and feature extraction and Dr. Globisch discussed metabolite identification. During the hands-on session, participants performed metabolite extraction from various sources, MS-based profiling, statistical, bioinformatic analysis and metabolite identification under the supervision Dr. Manna, who walked them through nuances of MS-based metabolomics in an interactive manner.



Snapshot from one of the interactive data analysis sessions during workshop.

SSABMS 2019 and Metabolomics Workshop at SINP, Kolkata

The event covered various applications of MS in proteomics, metabolomics, lipidomics, flux analysis, volatomics, microbiology, imaging and single cell analysis in the context of diseases including cancer, cardiovascular diseases, infectious diseases and inborn error of metabolism ranging from fundamental research to diagnostics. The breadth and depth along with ample opportunity to interact and network with the experts were widely appreciated by the participants. The first-hand exposure to the nuanced art of metabolomics during the workshop was very well-received. One of the aims of the event was to foster collaborative endeavours between researchers and clinicians to harness the prowess of MS in biomedical research and it has already started yielding initial results. The organizers deeply appreciate the enthusiastic response and look forward to organize such events regularly to serve the community.

Proteomics Society, India Registered under Andhra Pradesh Societies Registration Act. (35/2001) (Regd. No. 286/2009) proteomicsociety@ccmb.res.in www.psindia.org