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CURRICULUM VITAE

Present Position: Senior Principal Scientist and
DBT/Wellcome Trust India Alliance Senior Fellow
Institute of Microbial Technology
Sector 39 A, Chandigarh 160 036
&
Professor at Academy of Scientific & Innovative Research-IMTech

Major Scientific Achievements

1. **Establishing that pulmonary tuberculosis is biofilm infection.** I have demonstrated that intracellular thiol reductive stress induces mycobacterial biofilm formation. We have further demonstrated that cellulose is a major component of the extra polymeric substance of Mycobacterial biofilms and it degradation results in disruption Mycobacterial biofilms (*Nature Communications* 2016, 7: 11392). Before this study, mycolic acids were believed to be the most important constituents of the mycobacterial biofilms. Since cellulose is not present in the human bodies, it could be exploited for detection of the mycobacterial biofilms inside humans/animal models (Microbial Cell 2019). Thus, we have used cellulose as marker of biofilms lung tissue samples from mice and non-human primates. We have also detected the presence of mycobacterial communities inside the clinical human lung tissues. Furthermore, using mice model of TB, we have discovered that biofilms protect mycobacterial cells from the host immune system and is an important virulence factor. We have provided proof of concept data to suggest that cellulase could be used to enhance the activity of antimycobacterial activity in vivo [Nature communications 2021, Impact factor 12.12]. These findings were highlighted by Science (Science 02 Apr 2021:Vol. 372, Issue 6537, pp. 44-45. DOI: 10.1126/science.372.6537.44-c) and Nature Communications .

2. **Delineating the signaling pathway used by interferon gamma to induce autophagy.** Interferon gamma (IFNG) induces autophagy in macrophages to eliminate intracellular pathogens however the molecular mechanism was poorly understood. We have demonstrated that IFNG exposure induces increased levels of intracellular calcium in a HO-1 dependent manner. Increased calcium induces phosphatase calcineurin that dephosphorylates TFEB. Dephosphorylated TFEB moves into nucleus to induce autophagy and lysosomal biogenesis that play critical role in restriction of growth of intracellular *Mycobacterium tuberculosis*

(*Autophagy* 2018, DOI: 10.1080/15548627.2018.1436936). We believe that this pathway is critical for clearance of intracellular pathogens and viruses by IFNG.

3. **Establishment of SenX3-RegX3 two component system as oxygen regulated replication switch of Mycobacterium.** This study leads to a paradigm shift in the current understanding of oxygen controlled mycobacterial replication and provides evidence to suggest that the virulence factor SenX3 is a heme-based oxygen sensor that controls mycobacterial replication of persistent Mtb in response to oxygen (*Antioxidants & redox signaling* 2015, 22(7): 603-613). This study provides a plausible mechanism of oxygen-controlled reaction of TB disease in humans.
4. **Creation of tools to improve understanding of the physiology of intracellular Mtb.** My laboratory has adapted the genetically encoded biosensor Peredox to measure the NADH:NAD^+ ratio of Mtb *in vitro* and during the infection of macrophage cells. The nominee has demonstrated that the Mtb cells residing in macrophages display a metabolic heterogeneity, explaining the intracellular Mtb tolerance. They have shown that the activation of resting macrophages with interferon-gamma results in higher NADH:NAD+ levels in resident Mtb cells [**Frontiers in Cellular and Infection Microbiology 2016** Impact factor 3.5]. **In addition, they have made these reporter strains are readily available for use and distributed this resource to more than 50 laboratories across the world.** Furthermore, I am aware that the nominee's laboratory has also developed a new tool for measuring ATP/ADP with cellular resolution (manuscript under consideration) with subcellular resolution. I am of the view that these tools will be instrumental in our understanding of mycobacterial physiology and will open up new avenues of research. These tools could also lead to discoveries such as new metabolic pathways that may be unique to Mtb and the discovery of metabolic gradient across the mycobacterial cells.
5. **Mapping the age associated changes in mice lung microbiome.** We have mapped the changes in composition and diversity of the lung microbiome during developmental ages in mice. We have observed that the diversity of the lung microbiome increases during ages of 2-3 weeks and 5-6 weeks in the mice (*Microbiome* 2017, **5**:61 https://doi.org/10.1186/s40168-017-0277-3). This coincides with the age at which the alveolarization takes place.

EMPLOYMENT :

Year	Title	Institution/Department
June 2019-till date	Senior Principal Scientist	Institute of Microbial Technology Sector 39 A, Chandigarh 160 036 India
June 2014-May 2019	Principal Scientist	Institute of Microbial Technology Sector 39 A, Chandigarh 160 036 India

June 2010-May 2014	Senior Scientist	Institute of Microbial Technology Sector 39 A, Chandigarh 160 036 India
June 2009-May 2010	Instructor	Department of Microbiology, University of Alabama at Birmingham, Birmingham Alabama-35205.
Dec (2004)-June (2009)	Post-doctoral fellow	Department of Microbiology, University of Alabama at Birmingham, Birmingham Alabama-35205.
March-Aug (2004)	Post-doctoral fellow	Northwestern University Evanston, Fienberg School of Medicine, Evanston Illinois-60201
July 2002-Nov 2004	Senior Research Fellow	Counsel for Scientific and Industrial Research (Ministry of Human Resources and Development, Govt. of India), at Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi – 110007, India
July 2000-June 2002	Junior Research Fellow	Counsel for Scientific and Industrial Research (Ministry of Human Resources and Development, Govt. of India), at Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi – 110007, India

EDUCATION:

Ph.D. (2005) Biomedical Science	Under supervision of Prof. Vani Brahmachari (Dr. B. R. Ambedkar Center for Biomedical Research) and Prof. Mridula Bose (Vallabh Bhai Patel Chest Institute). Thesis: "An analysis and functional characterization of mammalian cell entry operons of *M. tuberculosis*" (University of Delhi).
M.Sc.Hons (2000) Biomedical Sciences	Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi – 110007, India
B.Sc. Hons 1998 (Microbiology)	Ram Lal Anand College, University of Delhi, Delhi- 110049, India

Professional Activities:

Academic Editor in Infection Biology section of Microbial Cell since 2016.

Life member of Society of Biological Chemists, India.

AWARDS:

- DBT/Wellcome Trust India Alliance Senior Fellowship (2021 to 2026).
- National Bioscience Award for Career Development (2017&2018) by Department of Biotechnology.
- Swarnajayanti Fellowship (2016-2017) in Life Sciences by Department of Science and Technology.
- Australia India Strategy Research Fund Awards for grand challenges sponsored through DST. 2013. Project Title "A Point of care diagnostic tool for tuberculosis".
- IKP Grand Challenges Explorations (2015) funded by the Bill and Melinda Gates Foundation. This award was received along with Dr Ashish Ganguly and Dr Amin Sagar for "XS-CATCH-TB: Detection of Mtb infection by X-ray scattering of hair and nail"
- UAB Centre for Free Radical Biology Travel Award for 14th Annual Society for Free Radical Biology and Medicine Meeting (SFRBM) in Washington, DC (2007).
- First prize for research presentation at UAB Postdoctoral Research Day, Feb, 2008.
- Excellence Award for postdoctoral research performed in the Department of Microbiology at University of Alabama at Birmingham.
- Keystone Symposia Scholarship for “Tuberculosis: From Lab Research to Field Trials” held in Vancouver, British Columbia, Canada (2007).

PUBLICATIONS:

1. Ajit Kumar Akela and **Ashwani Kumar**. Bioenergetic heterogeneity in Mycobacterium tuberculosis residing in different subcellular niches. **mBio**. 2021. June 1. ;e0108821 DOI: https://doi.org/10.1128/mBio.01088-21
2. *Poushali Chakraborty, Sapna Bajeli, Deepak Kaushal, Bishan Dass Radotra and **Ashwani Kumar**. Biofilm formation in the lung contributes to virulence and drug tolerance of Mycobacterium tuberculosis. **Nature Communications**. 2021. March 11, DOI: 10.1038/s41467-021-21748-6. (Impact Factor 12.12).
3. Iram Khan Iqbal, Sapna Bajeli, Shivani Sahu, Shabir Ahmad Bhat and **Ashwani Kumar**. Hydrogen sulfide-induced GAPDH sulfhydration disrupts the CCAR2-SIRT1 interaction to initiate autophagy. **Autophagy**. 2021 Jan 30:1-19. doi: 10.1080/15548627.2021.1876342.
4. Sapna Bajeli, Navin Baid, Manjot Kaur, Ganesh Popat Pawar, Vinod Chaudhari, **Ashwani Kumar**. 2020. Terminal respiratory oxidases: A targetable vulnerability of mycobacterial

bioenergetics? **Frontiers in Cellular and Infection Microbiology**. 2020. 10, 705. DOI: 10.3389/fcimb.2020.589318.

5. Parminder Singh Mavi, Shweta Singh and **Ashwani Kumar**. 2020. Reductive Stress: New Insights in Physiology and Drug Tolerance of Mycobacterium. Antioxidants and Redox Signaling. Antioxidants & Redox Signaling. 2020 Jun;32(18):1348-1366. DOI: 10.1089/ars.2019.7867. Epub 2019 Nov 19.
6. Poushali Chakraborty and **Ashwani Kumar**. 2019. The extracellular matrix of mycobacterial biofilms: could we shorten the treatment of mycobacterial infections? **Microbial Cell.** 2019. 6 (2), 105-122.
7. Naveen Thakur, **Ashwani Kumar**, Kanak L Dikshit. Type II flavohemoglobin of Mycobacterium smegmatis oxidizes d-lactate and mediate electron transfer. **International Journal of Biological Macromolecules**. 2018. 112: 868-875.
8. Nisha Singh, Zeeshan Ahmad, Navin Baid, **Ashwani Kumar**. Host heme oxygenase-1: Friend or foe in tackling pathogens? **IUBMB Life**. 2018. DOI: 10.1002/iub.1868.
9. Nisha Singh, Pallavi Kansal, Zeeshan Ahmad, Navin Baid, Hariom Kushwaha, Neeraj Khatri, **Ashwani Kumar**. Antimycobacterial effect of IFNG (interferon gamma)-induced autophagy depends on HMOX1 (heme oxygenase 1)-mediated increase in intracellular calcium levels and modulation of PPP3/calcineurin-TFEB (transcription factor EB) axis. **Autophagy**. **2018**. DOI: 10.1080/15548627.2018.1436936.
10. Iram Khan Iqbal, Sapna Bajeli, Ajit Kumar Akela, **Ashwani Kumar**. Bioenergetics of Mycobacterium: An emerging landscape for drug discovery. **Pathogens**. 2018. 7 (1). 1-24
11. Shabir Ahmad Bhat, Iram Khan Iqbal, **Ashwani Kumar**. Quantification of the Metabolic Heterogeneity in Mycobacterial Cells through the measurement of the NADH/NAD+ ratio using a genetically encoded sensor. Humana Press, New York, NY. **Cellular Heterogeneity** (2018). Chapter 14. 261-275.
12. Nisha Singh, Asheema Vats, Aditi Sharma, Amit Arora, **Ashwani Kumar**. The development of lower respiratory tract microbiome in mice. **Microbiome.** 2017, 5**:**61 https://doi.org/10.1186/s40168-017-0277-3.
13. Shabir Ahmad Bhat, Iram Khan Iqbal and **Ashwani Kumar**. Imaging the NADH:NAD+ homeostasis for understanding the metabolic response of Mycobacterium to physiologically relevant stresses**. Frontiers in Cellular and Infection Microbiology**. 6:145 doi: 10.3389/fcimb.2016.00145.
14. Vijjamarri Anil Kumar, Rajni Goyal, Roohi Bansal, Nisha Singh, Ritesh Rajesh Sevalkar, **Ashwani Kumar**, Dibyendu Sarkar. EspR-dependent ESAT-6 protein secretion of

Mycobacterium tuberculosis requires the presence of virulence regulator PhoP. **Journal of Biological Chemistry** 2016, 2016, 291(36):19018-30. doi: 10.1074/jbc.M116.746289.

15. Nupur L. N. U., Asheema Vats, Sandeep Kumar Dhanda, Gajendra P. S. Raghava, Anil Kumar Pinnaka and **Ashwani Kumar**. ProCarDB: a database of bacterial carotenoids. **BMC Microbiology** 2016, 16:96 DOI: 10.1186/s12866-016-0715-6.
16. Piyush Baindara, Nisha Singh, Manish Ranjan, Nayudu Nallabelli, Vasvi Chaudhry, Geeta Lal Pathania, Nidhi Sharma, **Ashwani Kumar**, Prabhu B Patil, Suresh Korpole. Laterosporulin10: a novel defensin like Class IId bacteriocin from Brevibacillus sp. strain SKDU10 with inhibitory activity against microbial pathogens. **Microbiology** 2016, 162, 1286–1299.
17. **Ashwani Kumar**. House of cellulose–a new hideout for drug tolerant Mycobacterium tuberculosis. **Microbial Cell**. 2016, 3 (7) 299-301.
18. Abhishek Trivedi, Parminder Singh Mavi, Deepak Bhatt & **Ashwani Kumar**. Thiol reductive stress induces cellulose-anchored biofilm formation in Mycobacterium tuberculosis. **Nature communications** 2016 Apr 25;7:11392. doi: 10.1038/ncomms11392.
19. Nisha Singh and Ashwani Kumar. Virulence factor SenX3 is the oxygen-controlled replication switch of Mycobacterium tuberculosis. **Antioxidants & redox signaling** 2015, 22(7): 603-613
20. Deepak Singla, Rupinder Tewari, **Ashwani Kumar**, Gajendra Ps Raghava. Designing of inhibitors against drug tolerant Mycobacterium tuberculosis (H37Rv). **Chemistry Central Journal** 03/2013; 7(1):49.
21. Vemika Chandra, Sahil Mahajan, Ankita Saini, Hedwin K Dkhar,Ravikanth Nanduri, Ella B Raj, **Ashwani Kumar**, Pawan Gupta. Human IL10 repression by Reverb alpha ameliorates_Mycobacterium_tuberculosis clearance. **Journal of Biological Chemistry**. 2013.
22. Shabir Ahmad Bhat, Nisha Singh, Abhishek Trivedi, Pallavi Kansal, Pawan Gupta and **Ashwani Kumar**. The mechanism of redox sensing in Mycobacterium tuberculosis. **Free Radic Biol Med**. 2012 Oct 15;53(8):1625-41. doi: 10.1016/j.freeradbiomed.2012.08.008. Epub 2012 Aug 11.
23. Abhishek Trivedi, Nisha Singh, Shabir Ahmed Bhat, Pawan Gupta and **Ashwani Kumar**. Redox Biology of Tuberculosis Pathogenesis. **Advances in microbial physiology**. 2012;60:263-324.
24. Aisha Farhana, Vikram Saini, **Ashwani Kumar**, Jack R Lancaster Jr and Adrie JC Steyn Environmental heme-based sensor proteins: Implications for understanding bacterial pathogenesis. **Antioxidants and redox signaling**. 2012, 17(9) 1232-1245.

25. Sandeep Dave, Naval Jit Kaur, Ravikanth Nanduri, H Kitdorlang Dkhar, **Ashwani Kumar**, Pawan Gupta.Inhibition of Adipogenesis and Induction of Apoptosis and Lipolysis by Stem Bromelain in 3T3-L1 Adipocytes. **PLoS One.** 2012;7(1):e30831. Epub 2012 Jan 24.
26. **Ashwani Kumar,** Aisha Farhana, Loni Guidry, Vikram Saini, Mary Hondalus, Adrie JC Steyn. Redox homeostasis in mycobacteria: the key to tuberculosis control? **Expert Rev Mol Med.** 2011 Dec 16;13:e39.
27. † **Ashwani Kumar,** Jessy Deshane, David Crossman, Subhashini Bolishetty, Bo-Shuin Yan, Igor Kramnik, Anupam Agarwal and Adrie J. C. Steyn. Heme oxygenase-1 derived carbon monoxide induce the *Mycobacterium tuberculosis* dormancy regulon. **Journal of Biological Chemistry.** 2008. 283 (26) 18032–18039.
28. ‡ **Ashwani Kumar**, Jose C. Toledo, Rakesh P. Patel, Jack Lancaster Jr, and Adrie J. C. Steyn. *Mycobacterium tuberculosis* DosS is a redox sensor and DosT is a hypoxia sensor. **Proceedings of National Academy of Sciences.** 2007. 108 (28) 11568-11573. *Reviewed by the Faculty of 1000.*
29. € Amit Singh, Deborah Mai, **Ashwani Kumar** and Adrie J. C. Steyn. Dissecting virulence pathways of *Mycobacterium tuberculosis* through protein-protein association. **Proceedings of National Academy of Sciences**. 2006. 130 (30) 11346-11351.
30. **Ashwani Kumar**, Amita Chandolia, Uma Chaudhry, Vani Brahmachari, Mridula Bose. Comparison of mammalian cell entry operons of mycobacteria: *in silico* analysis and expression profiling**. FEMS Immunology Medical Microbiology**. 2005. 43 (2):185-195.
31. **Ashwani Kumar**, Mridula Bose and Vani Brahmachari. Analysis of Expression profile of mammalian cell entry (mce) operons of *Mycobacterium tuberculosis*. **Infection and Immunity.** 2003. 71 (10): 6083-6087.
32. Mandira Verma, Sujit Kumar, **Ashwani Kumar** and Mridula Bose. Comparison of E-test and agar proportion method of testing susceptibility of *M. tuberculosis*. **Indian Journal of Tuberculosis.** 2002. 49 (4): 217-220.

* This article featured as the **Editor's choice of the week in Science**. Science 02 Apr 2021: Vol. 372, Issue 6537, pp. 44-45. DOI: 10.1126/science.372.6537.44-c. This article was also picked up by Nature Communication for the Editors' Highlights webpage of recent research called "Microbiology and infectious diseases".

† This article first time described the role of carbon monoxide in regulation of tuberculosis dormancy program and has received overwhelming media response. More details can be obtained at the following link.

http://www.google.com/search?hl=en&q=tuberculosis+carbon+monoxide+news

This article appeared in news of Cell Host and Microbes 2008, 3 (5) 277-279.

This article also appeared in research highlight of ***Nature Reviews Microbiology*** 2008, **6**: 501 - 501.

‡ This manuscript represents the first report of the mechanisms used by Mycobacteria to sense hypoxia, nitric oxide, and carbon monoxide. This manuscript featured as an important article by Faculty of 1000 Biology and was reviewed independently by two members.
€ This manuscript demonstrated, the development Mycobacterial protein fragment complementation (M-PFC), as an effective method for exploring *Mtb* protein–protein interactions in Mycobacteria. This manuscript was featured by Faculty of 1000 Biology, in addition of appearing in **spot light** of Journal "**chemical biology**" (http://pubs.acs.org/cgi-bin/abstract.cgi/acbcct/2006/1/i08/abs/cb600392e.html).

PATENTS:

1. **Adrie J. C. Steyn and Ashwani Kumar.** Modulating latency and reactivation of *Mycobacterium tuberculosis*. US Patent application number: 20100239691. IPC8 Class: AA61K3300FI; Publication date: 09/23/2010.
2. **Ashish, Ashwani Kumar, Amin Sagar**. In vitro method for detecting active mycobacterium tuberculosis using hair small angle x-ray scattering profile. Application number: PCT/IN2017/050547 and WO2018096557A1

GRANT SUPPORT: Dr Ashwani Kumar has been supported by the following extra-mural grants

1. BT/PR15097/MED/29/237/2011: DBT sponsored project with the title "Role of HO-1 and its reaction products in modulation of TB pathogenesis"
Project Status: completed.
Funding amount: INR 59 Lakhs
Project Summary: Recently published studies have suggested that the virulent/pathogenic strains of mycobacteria significantly inhibit apoptosis of infected macrophages cells and hence are able to inhibit efficient activation of adaptive immune system. However infection of macrophage with avirulent/non-pathogenic strains of mycobacteria leads to initiation of efficient apoptosis and thus activation of the adaptive immune system. The molecular mechanism utilized by virulent mycobacteria to inhibit apoptosis is unknown. Our recently published work has established that Mtb infection of macrophage cells leads to increased heme oxygenase-1 transcript, protein and activity levels. Heme oxygenase 1 and its reaction products are well established potent inhibitors of apoptosis. Hence this proposal is focused at analyzing the role of heme oxygenase-1 in inhibition of apoptosis of infected macrophage cells and in TB pathogenesis. This first part of this proposal is aimed at understanding the differences in HO-1 sub-cellular localization and activity upon infection with pathogenic and non-pathogenic mycobacteria. These differences will be then correlated to apoptosis of infected macrophages. This part of the application will test whether HO-1 is the key modulator that leads to apoptosis in response to infection with avirulent mycobacteria and inhibits

apoptosis in response to infection with virulent mycobacteria. The second part of this project will analyze the role of HO-1 and its reaction products in TB pathogenesis. In this part of the application we propose to utilize the HO-1 knockout mice strains instead of HO-1 inhibitors that often lead to non-specific reactions. The experiments proposed in this application will incisively dissect the role of HO-1 in TB pathogenesis. Since HO-1 could be pharmacologically modulated, the findings of this application may have translational applications and represents a unique aspect of this study.

2. BT/PR/5086/GBD/27/307/2011: DBT sponsored project with the title ""Analysis of role of mycothiol and thioredoxin in maintaining redox homeostasis of mycobacteria using novel RoGFP based redox measuring tool"

Project Status: completed.

Funding amount: INR 25.5 Lakhs

Project Summary:

Mycobacterium is exposed to a number of destructive redox active molecules during infection. The success of mycobacteria as a pathogen is dependent on its ability to sense and resist host generated oxido-reductive stress during infection. A clear understanding of molecular mechanisms utilized by Mtb to sense changes in the redox environment and to maintain redox homeostasis in *Mtb* is limited, primarily due to lack of a non-invasive tool, capable of directly measuring intracellular redox potential. In this application, we propose to creatively develop a novel non-invasive tool. This tool will exploit recently engineered redox sensitive variant of green fluorescent protein (RoGFP) to quantify the intracellular redox environment of mycobacteria in real time. This tool will be utilized for measuring the mid-point potential for pathogenic and non-pathogenic species of mycobacterium. We will further utilize this tool for dissecting the role played by mycothiols and thioredoxins in the redox homeostasis of mycobacterium. For analysis of role of mycothiols in redox homeostasis, we will utilize the genetic mutants in the mycothiol biosynthetic pathway and the mid-point potential of these mutants will be measured. For analysis of role of thioredoxins in redox homeostasis we will utilize a *sigH* mutant since *sigH* regulates the expression of thioredoxins. We will further validate our data using the specific chemical inhibitors of thioredoxin reductase (the enzyme that reduces the oxidized thioredoxins). The use of novel RoGFP based tool along with the employment of genetic and chemical approach for the analysis of role of mycothiols and thioredoxins in redox homeostasis will be unique and distinguishing aspect of the proposed study.

3. DST/INT/AUS/GCP-7/13: Australia India Strategy Research Fund Awards for grand challenges sponsored through DST. Title of the project is "A point-of-care diagnostic tool for tuberculosis".

Project Status: The project is under progress and research work is going on the same.

Funding amount: INR ~ 190 Lakhs

Project Summary: Tuberculosis is one of the leading causes of mortality and morbidity worldwide, killing 1.7 million people annually. Presently only 65% of estimated TB cases are reported while the remaining 35% goes undiagnosed and untreated suggesting an urgent need for developing novel diagnostic tools to restrain the chain of tuberculosis transmission. We intend to develop a simple, robust and cost effective technology that could be used at community level. The proposed technology employs robust cost effective sensory modules using chemiresistor technology attached to smart microcontrollers, so the data analysis could be visualized and clinical decision support provided using the processing capability of an ordinary mobile phone, the type of phone found extensively among health care workers in resource poor settings. Hence the technology holds promise for taking the diagnosis to the user (point of care) with the additional opportunity of having data analyzed remotely for both diagnosis and surveillance purposes. The sensor component is ink-jet printable and hence extremely robust, miniaturisable and inexpensive to produce. The advent of such a technology would increase the rate of TB detection and thus increase the number of treated patients thereby significantly disrupting the chain of TB transmission. The proposed method is dependent on recognition of a complex but specific molecular signature in fluid samples of TB patients. Since this molecular signature could result from either the unique bacterial components or from a specific host response to the infection (or both), this technology holds promise for detection of extra-pulmonary TB, pediatric TB and TB in HIV infected individuals as well. Such a technology is also highly desirable for biosecurity purposes and in monitoring the level of Tb among immigrants.

4. SR/SO/BB-0037/2013: DST funded project with title "Deciphering the role of heme-binding sensor kinase SenX3 and its paired response regulator RegX3 in physiology of *Mycobacterium tuberculosis*"

Project Status: Project completed.

Funding amount: INR ~ 36 Lakhs

Project Summary: Two component system proteins, serine/threonine kinases along with the one component systems constitute signal sensing machinery of *Mycobacterium tuberculosis*. However the mechanisms of signal sensing and gene regulation thereafter are poorly understood. In the present proposal we intend to delineate the molecular mechanism of diatomic gas sensing by histidine kinase protein SenX3. We further propose to decipher the effect of the diatomic gas binding with SenX3 on its autokinase activity. We also propose to decipher the role of RegX3 in latency and reactivation of *Mycobacterium tuberculosis* using a number of biochemical and molecular biology techniques. The methods used in this proposal will establish the pathways directly regulated by RegX3. The findings

of this proposal will significantly enhance our understandings of physiological functioning of SenX3-RegX3 two component system in *Mycobacterium tuberculosis*.

5. HCP0001B: OSDD-CSIR sponsored Project with title "Mapping the cross-talks between sensor kinases and response regulators using mycobacterial protein fragment complementation"
Project Status: Project completed.
Funding amount: INR ~ 25 Lakhs
Project Summary: The aim of this project was to map the cross-talks between sensor kinases and response regulators using mycobacterial protein fragment complementation. Towards this, MPFC was used and an intricate network of cross talks was delineated.

6. DST/SJF/LSA-02/2016-2017: DST sponsored Project with title "Unraveling the cellulose biosynthesis machinery and deciphering its role in mycobacterial biofilms"
Project Status: Ongoing Project completed.
Funding amount: INR 125 Lakhs

7. BTIHRD-NBA-NWB/37/01I2018: DBT sponsored Project with title "Development of reporter strains of *Mycobacterium tuberculosis* for measurement of ATP:ADP with spatiotemporal resolution to probe metabolic heterogeneity during infection"
Project Status: Ongoing Project completed.
Funding amount: INR 15 Lakhs

8. BT/PR30745/MED/29/1366/2018: DBT sponsored Project with title "The Role of Hydrogen Sulfide in Modulation of Autophagy and Tuberculosis Pathogenesis"
Project Status: Ongoing Project completed.
Funding amount: INR 85 Lakhs

9. IA/S/20/2/505220: DBT/Wellcome Trust India Alliance sponsored project with title "Relevance of Mycobacterium tuberculosis biofilms in tuberculosis pathogenesis and drug tolerance"
Project Status: Ongoing Project completed.
Funding amount: INR 445 Lakhs

Ph.D. Students trained:

Sl. no	Student name	Thesis Title
1	Nisha Singh	Studies on SenX3-RegX3 two component signal transduction system and the role of carbon monoxide in tuberculosis pathogenesis
2	Abhishek Trivedi	Exploring the Phenomenon of quorum sensing and biofilm formation in Mycobacterium tuberculosis
3	Shabir Ahmad Bhat	Understanding the redox biology of tuberculosis pathogenesis using genetically encoded sensors
4	Zeeshan Ahmad	The role of heme oxygenase-1 & its reaction products in the modulation of cellular signaling: New insights in tuberculosis pathogenesis
5	Iram Khan	Deciphering the role of hydrogen sulfide in cellular signaling
6	Naveen Thakur	Molecular characterization of Mycobacterial falvohemoglobins
7	Parminder Singh Mavy	Utilizing high resolution microscopy for understanding the physiology of *Mycobacterium*
8	Ajit Kumar Akela	Utilizing genetically encoded sensors for understanding the cellular physiology during mycobacterial infections
9	Poushali Chakraborty	Exploring the nature of extracellular polymeric substances in mycobacterial biofilms and role of microbiome in tuberculosis pathogenesis
10	Naveen Baid	Exploring the subcellular localisation of Mycobacterium in infected macrophages

TEACHING EXPERIENCE:

- Since 2010, Faculty (Associate Professor) in Jawaharlal Nehru University-Institute of Microbial Technology graduate program.
- Professor in the ACSIR-IMTech Ph.D program.
- Coordinator of Term paper examination in this graduate program.
- Academic activities undertaken as a Senior Scientist at CSIR-IMTech;
- Coordinator of “Tuberculosis advanced course work under ACSIR-IMTech Ph.D program. Besides I am also Coordinator of “Infectious Disease Journal club” at IMTech.

Outreach programmes:

- Coordinated summer research training program as a part of Science outreach program titled “Jigyasa 2015”, for students from neighbouring schools. The ideology behind Jigyasa'15 was

to introduce the school students to the scientific research process with an emphasis on asking questions, making observation, experimentation, data analyses and record keeping.

- Coordinator of Science outreach program of IMTech, Chandigarh. see more details at the website; http://www.imtech.res.in/StudentOutreach/
- Scored oral presentation of graduate students of UAB as a judge on the Annual Graduate Research Day Feb 29th 2008.
- Scored poster of graduate students (Cellular and Molecular Biology program) as a judge on Feb 12th 2008 at UAB.
- Guided Science and Technology students (a high school student) for a three-month project in order to explore the cutting edge research technology used in the field of tuberculosis. 2006.
- Training high school student, Kaitlyn to explore cutting edge techniques in Molecular Biology in six months project. 2007.
- Training in basic techniques in Molecular Biology, for International scholar (Bass from Mali) 2007.

INVITED TALKS (Selected)

- Infection and Immunology seminar series (lecture delivered online) at the Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden. 14th June 2021. Presentation title: Cellulose: key to mycobacterial drug tolerance and virulence?
- International Conference on Infectious Diseases and Immunopathology (Online) organized by Department of Biotechnology, Savitribai Phule Pune University. 22nd to 24th April 2021. Presentation title: Cellulose: key to mycobacterial drug tolerance and virulence?
- Invited lecture at Department of Microbiology & Immunology Albert Einstein College of Medicine. Invitation by William Jacobs. March 18th 2021. Presentation title: Mycobacterial biofilms: relevance to virulence and drug tolerance during infection
- NIPER-Hyderabad scientific webinar on World TB day on 24 March 2021. Presentation title: Mycobacterial biofilms: relevance to virulence and drug tolerance during infection.
- National conference (online) on Host-pathogen interaction: Present and future perspectives. Organized by Department of Life Science, National institute of Technology, Rourkela, Odisha 24th & 25th September, 2020. Presentation title: Diatomic gases modulate trafficking and intracellular survival of Mycobacterium tuberculosis".
- India/EMBO Symposium on Mycobacterial Heterogeneity and host tissue tropism at National Institute of Immunology and International Center for Genetic Engineering, New Delhi 11 to 15th

Feb 2020. Presentation title: Hydrogen Sulfide-Induced GAPDH Sulfhydration Disrupts the DBC1-Sirt1 Interaction to Initiate Autophagy and Restricts the mycobacterial growth.

- International Conference on Autophagy and Lysosomes at Division of Biological Sciences, Indian Institute of Sciences, Bengaluru. 18-18th January 2020. Presentation title: Hydrogen Sulfide-Induced GAPDH Sulfhydration Disrupts the DBC1-SIRT1 Interaction to Initiate Autophagy.
- GE Healthcare India Microscopy User Workshop at Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru. 24 -25th September 2019. Presentation title: Hydrogen sulfide-induced GAPDH Sulfhydration disrupts the DBC-1 Sirt-1 interaction to initiate autophagy.
- Molecular Microbiology Meeting. Second M^3 meeting at Center for DNA Fingerprinting, Hyderabad. July 10-12th 2019. Presentation title: “Mycobacterial biofilms: are they relevant in vivo?”
- Biological Transactions: From Molecules to Organisms (2019) at Indian Institute of Sciences, Bengaluru. January 17-20th 2019. Presentation title: “Hydrogen sulfide induced GAPDH sulfhydration disrupts DBC-Sirt1 interaction to initiate autophagy”
- India Autophagy Core Group Meeting at Translational Health Science and Technology Institute, Faridabad during 10-11 August 2018. Presentation title: Hydrogen sulfide induced GAPDH sulfhydration disrupts DBC-Sirt1 interaction to initiate autophagy.
- Short talk in India, EMBO symposium titled “Autophagy: Cellular mechanisms and significance in health and disease” organized at Bhubaneswar during 11-13 December 2017. Presentation title: Understanding the Mechanisms of Regulation of Autophagy by Signaling Gases Hydrogen Sulfide and Carbon Monoxide.
- Mini-Symposium on cell biology at National Centre for Cell Science , Pune held on 26th October 2017. Presentation Title: Understanding the nature of extracellular polymeric substances of the Mycobacterium tuberculosis biofilms
- International conference titled “Microbiology in the new Millennium : from Molecules to Communities” (MNM 2017) held during 27 to 29 October, 2017 at the new Unified Campus of Bose Institute, Kolkata. Presentation title: Cellulose is a biomarker of biofilms of Mycobacterium tuberculosis biofilms.
- Dr. Paul Jenssen TB Sympossium at CSIR-IMTech, Chandigarh on 17th August 2017. Presentation title: Mycobacterial bioenergetics; A novel target space.
- EMBO conference on Bacterial Morphogenesis, Survival and Virulence: Regulation in 4D held from the 27th of November - 1st of December 2016 in Thiruvananthapuram, Kerala, India.

Presentation title: House of cellulose - a new hideout for drug tolerant Mycobacterium tuberculosis.

- Scientific session in the Silver Jubilee Celebrations of foundation of Dr B. R. Ambedkar Center for Biomedical Research, University of Delhi. 14th April 2016. Presentation Title "Heme oxygenase-1 mediated control of autophagy; its implication in tuberculosis pathogenesis".
- International Conference on New Horizons in Biotechnology (NHBT-2015). November 22-25, 2015. Trivandrum, Kerala, India. Presentation Title "Phenotypic switching in Mycobacterium tuberculosis".
- Symposium on Frontiers in Biomedical Research held at University of Delhi. 29-31st Oct 2015. Presentation Title "Where is the drug resistance hiding; lookout in the house of cellulose".
- Symposium on Emerging themes in tuberculosis research; creating a network. Held at Indian Institute of Sciences, Bangalore. July 18-23 2013. Presentation title Oxygen sensing in regulation of mycobacterial latency and reactivation.
- Indo-Canada symposium on “Redox Status and Control in TB: From Basic Research to Drug Development”, January 30th to February 1st, 2011, Hyderabad. Lecture title "emerging mechanisms of drug resistance in Mycobacterium.
- 108th General Meeting of American Society of Microbiology in Boston, June 1-5 2008. Section 198/U. New perspectives on mechanisms of redox sensing by *Mycobacterium tuberculosis*. Sniffing protective host signals through Fe-S and heme-based sensor proteins
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SELECT ABSTRACTS:

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- 70th Annual Meeting of Society of Biological Chemists, Hyderabad, India. 27-29 December 2001. "Expression analysis of mammalian cell entry (mce) operons of *M. tuberculosis"* Ashwani Kumar, Mridula Bose and Vani Brahmachari.
- First Annual Symposium in Biomedical Sciences, University of Delhi, April 2001. Analysis of sequence variations of mce operons in clinical strains of *M. tuberculosis*. **Ashwani Kumar**, Mridula Bose and Vani Brahmachari.