List of 10 best papers

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.

   Highlights: This manuscript describes development of a novel tool for tracking ATP/ADP levels at the single-cell and population levels. We have utilized this tool to demonstrate that the transcription inhibitor rifampicin induces metabolic stress while the cell wall synthesis inhibitor isoniazid does not alter the metabolic state of the bacilli, suggesting that transcription is tightly intertwined with metabolism, while cell wall synthesis is not. Furthermore, we analyzed the metabolic state of mycobacteria residing in different compartments of macrophages. We observed that Mtb cells residing inside phagosomes have healthy ATP/ADP levels. In contrast, the bacteria residing inside phagolysosomes and autophagosomes face depletion of ATP.

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).

   Highlights: Tuberculosis displays several features of a biofilm-associated infection, but if it is a biofilm infection is not known. This study for the first time demonstrated the formation of *Mycobacterium tuberculosis* (Mtb) biofilms in vivo. Using cellulose as a biomarker to detect Mtb biofilms, we demonstrated the presence of Mtb biofilms in the lungs of experimentally infected mice and non-human primates, as well as in lung tissue sections obtained from patients with tuberculosis. Mtb strains defective in biofilm formation were utilize to establish that biofilms protect bacilli from the host immune system. Furthermore, the administration of nebulized cellulase enhanced the antimycobacterial activity of isoniazid and rifampicin in infected mice, supporting a role for biofilms in phenotypic drug tolerance.
   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".

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.

   Highlights: Hydrogen sulfide (H_2S) imparts several health benefits but its role in TB pathogenesis has remained largely unexplored. This study has delineated the molecular mechanism of H_2S mediated autophagy. Briefly, H_2S induces nuclear localisation of glycolytic enzyme GAPDH. Nuclear GAPDH interacts with CCAR2/DBC1 (cell cycle activator a nd apoptosis regulator 2) and relieves its inhibitory effect on deacetylase Sirt1. This study further suggests that H_2S enables autophagy-mediated trafficking of Mtb into lysosomes to restrict intracellular mycobacterial growth. We think that the pathway described here could be involved in H_2S-mediated clearance of intracellular pathogens and other health benefits.

4. 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.

   Highlights: IFNG (interferon gamma) plays a critical role in controlling the *Mycobacterium tuberculosis* (Mtb) infection. However, the molecular basis of IFNG mediated killing of Mtb remains poorly defined. In this study we demonstrated that HMOX1 (heme oxygenase 1)-generated carbon monoxide (CO) is required for the induction of autophagy and killing of Mtb residing in macrophages in response to immunomodulation by IFNG. The essence of this study is that IFNG-mediated increase in intracellular calcium leads to activation of the phosphatase calcineurin (PPP3), which dephosphorylates the TFEB (transcription factor EB) to induce autophagy. PPP3-mediated activation and nuclear translocation of TFEB are critical in IFNG-mediated mycobacterial trafficking and survival inside the infected macrophages.

5. 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.

   Highlights: This manuscript describes the details of a method used in the generation of reporter strains for the measurement of the NADH/NAD+ ratio in mycobacteria. Importantly, once the reporter strains are created, they can be exploited with fluorescence spectroscopy, FACS, and confocal microscopy to access the dynamic changes in the NADH/NAD+ levels in intact individual bacterial cells.

6. 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.

   Highlights: The alveolarization of lung takes after birth in two phases, but whether these affect the microbiome of lung is not known. In this manuscript 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. This coincides with the age at which the alveolarization takes place.

7. 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.

   Highlights: The NADH:NAD+ ratio is the primary indicator of the metabolic state of bacteria. In this manuscript, we have adapted the genetically encoded biosensor Peredox for measurement of the metabolic state of Mtb in vitro and during infection of

macrophage cells. We demonstrated that the Mtb cells residing in macrophages display a metabolic heterogeneity, which may perhaps explain the tolerance displayed by intracellular Mtb. Next we showed that the activation of resting macrophages with interferon-gamma results in higher NADH:NAD+ levels in resident Mtb cells. These reporter strains are readily available for use. We have so far distributed this resource to ~30 laboratories across the world.

8. 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.

   Highlights: This manuscript describes the discovery that cellulose is a structural component of the Mycobacterial Biofilms. In this manuscript 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. 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, we have proposed that cellulose could be used as a biomarker for detection of the mycobacterial biofilms inside humans/animal models (Microbial Cell, 2019. 6(2) 105–122).
   Microbial cell has published a commentary on this manuscript.

9. 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

   Highlights: Morphogenetic switching between the replicating and nonreplicating states of Mycobacterium tuberculosis is regulated by oxygen, nitric oxide, and carbon monoxide levels, however, the mechanisms by which M. tuberculosis senses these diatomic gases remain poorly understood. In this study, we demonstrate that the virulence factor SenX3 is a heme protein that acts as a three-way sensor with three levels of activity. Importantly SenX3 mutant of M. tuberculosis was capable of attaining a nonreplicating persistent state in response to hypoxic stress, but its regrowth on the restoration of ambient oxygen levels was significantly attenuated compared with the wild-type and the complemented mutant strains. Evidence presented in this study delineates a plausible mechanism explaining the oxygen-induced reactivation of tuberculosis diseases in humans after many years of latent infection.

10. **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.*

   Highlights: Hypoxia and NO induce nonreplicating persistent state in Mycobacterium tuberculosis. In this study we demonstrated that DosS and DosT bind heme as a prosthetic group and that DosS is rapidly autooxidized to attain the met (Fe3+) form, whereas DosT exists in the O2-bound (oxy) form. EPR and UV-visible spectroscopy analysis showed that O2, NO, and CO are ligands of DosS and DosT. Importantly, we demonstrate that the oxidation or ligation state of the heme iron modulates DosS and DosT autokinase activity and that ferrous DosS, and deoxy DosT, show significantly

increased autokinase activity compared with met DosS and oxy DosT. Our data provide direct proof that DosS functions as a redox sensor, whereas DosT functions as a hypoxia sensor. We concluded that Mtb has evolved finely tuned redox and hypoxia-mediated sensing strategies for detecting O2, NO, and CO. This study established a paradigm for understanding the mechanism of bacilli persistence.
This manuscript featured as an important article by Faculty of 1000
Biology and was reviewed independently by two members.

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.