REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) Year 2018-19 in Training/Research abroad.

1. **Name and designation of ICMR- IF** :**Vivek Kumar Gupta, Scientist-D**

2. **Address** :Department of Biochemistry, ICMR-National JALMA Institute for Leprosy& Other Mycobacterial Diseases, Tajganj, Agra

3. Frontline area of research in which training/research was carried out :Mycobacterial Resuscitation Promoting factors

4. Name & address of Professor and host institute : Dr. Galina Mukamolova,

   Associate Professor, Department of Respiratory Sciences (previous name- Department of Infection, Immunity and Inflammation), University of Leicester LE1 7RH, UK

5. Duration of fellowship : Six months (2nd October2018 to 30th March 2019)

6. Highlights of work conducted :

i) Technique/expertise acquired :

ICMR-IF has acquired sufficient technical skills in Detection and characterization of resuscitation promoting factors using different techniques- Ammonium sulfate protein precipitation, Protein purification by Ion-exchange chromatography, SDS-PAGE, Western blotting, Colony PCR, Real Time PCR, purification of Rpf raised polyclonal antibody from animal sera, MPN counting for characterization of dormant bacilli population in sample etc.

ii) Research results, including any papers, prepared/submitted for publication

Tuberculosis (TB) is a curable disease but still remains one of the biggest killers in the world. It kills nearly 2 million people worldwide every year and 98% of tuberculosis deaths are in the developing world affecting mostly young adults in their productive years. One important characteristics of this disease is that the bacterium has an unusual ability to grow and survive for extended periods of time in human body. Therefore, it has been estimated that 2 billion people, equal to one third of the world's total population, are infected with the bacterium in whom it causes unnoticeable latent infection that gives rise to a 5-10% lifetime risk of active tuberculosis. These persistent bacteria cannot be cultured using standard microbiological methods and are not killed by the current tuberculosis drugs. Therefore, tuberculosis treatment needs at least 6 months with four drugs to cure the patients.

The persistent bacteria will be "woken" by the addition of resuscitation promoting factors (RPFs) which are proteins produced by *M. tuberculosis* (*Mtb*) to restart growth. The Rpf proteins, which are believed to have muralytic activity, are widely distributed throughout the actinobacteria, including *M. tuberculosis*, and they are implicated in the resuscitation of dormant forms of these organisms. Rpf proteins have clinical implications as they increase the recovery of *Mtb* from clinical samples of the patient with active TB and improved the sensitivity of culture-based *Mtb* tests (Mukamolova, 2010; Huang et al., 2014; Chengalroyen et al., 2016; Rosser, 2017). Moreover, Rpf-dependent *Mtb* are more resistant to antimicrobial agents and are extremely difficult to eradicate from patients (Turapov, 2016). Therefore, drugs specifically targeting non replicating persistence and Rpf-dependent *Mtb* are urgently needed (Kaprelyants et al., 2012).

Rpfs are widespread among Actinobacteria species with five homologues existing in *Mtb* (RpfA, RpfB, RpfC, RpfD, RpfE) and four (RpfA, RpfB, RpfC, RpfE) having been annotated in the closely related *Mycobacterium marinum. M. marinum* is increasingly being used to model TB infection. With a faster generation time of 12 hours, *M. marinum* is a close genetic relative of *M. tuberculosis* sharing 85 % nucleotide identity, and 3000 orthologs with an average amino acid identity of 85 % (Stinear et al., 2008). Key genes encoding proteins essential for the virulence and pathogenicity of *Mtb* are also conserved in *M. marinum* including the PE/PPE, ESX and Resuscitation promoting factor (Rpf) families.

Research Results: Considering the above facts, detection and expression of Rpf A was performed using *M. marinum* as model organism for this study. The detection of rpfA was carried out in the secretory proteins using different media. The culture conditions were standardized for secretory protein secretion. Sauton's Medium was found to be best medium for excretory protein yields. The two techniques TCA and ammonium sulfate precipitation were implemented for precipitation of proteins from culture filtrates.

Proteins was precipitated using 60 to 80% saturation of ammonium sulfate and purified through membrane dialysis but yields and amount were less. Ion exchange chromatography was used for protein purification with DEAE Sepharose. Ion exchange chromatography was found to be better technique for detection of secretory proteins through SDS-PAGE.

The RpfA was detected in *M. marinum* (WT) through immunoblotting. Purified proteins were analysed by SDS–PAGE. Coomassie brilliant blue was used to stain protein bands. Proteins were electroblotted onto nitrocellulose blotting membranes for western blot analysis. The polyclonal antibodies generated in rabbit against recombinant Rpf (1:10,000 dilution) was used as primary antibody for detection of RpfA. Secondary antibody was horseradish-peroxidase (HRP)-conjugated anti-rabbit IgG (1:10,000 dilution). The blot was detected under chemoluminance using substrate signal FireTM Elite ECL Reagent ((Fig. 1 & Fig. 2). RpfA secretory protein which was detected in culture filtrates of *M. marinum* (WT). The protocol for detection of RpfA from culture filtrates was developed during the study.