# REPORT

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

| | | |
|---|---|---|
| 1. | Name and designation of ICMR- IF | : **Dr. Yeshvandra Verma, Assistant Professor** |
| 2. | Address | : Department of Toxicology C.C.S.University, Meerut-250004 |
| 3. | Frontline area of research in which training/research was carried out | : Toxicology |
| 4. | Name & address of Professor and host institute | : Prof. Valentina A. Kratasyuk Institute of Fundamental Biology & Biotechnology, Siberian Federal University, Krasnoyarsk, Russia |
| 5. | Duration of fellowship with exact date | : **26 January 2020 to 23 May 2020** |

6. **Highlights of work conducted-**

**i)Technique/expertise acquired** :

**(a)** Learned the techniques to immobilize bioluminescent bacteria and their application in developing biosensor to detect the toxicity of xenobiotics.

**(b)** Developed a novel method for synthesis of silver nanoparticles using suitable media for toxicity assays.

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

**Results**

***Synthesis of nanoparticles***

Silver nanoparticles were synthesised using silver nitrate and sodium borohydride in common while the surface ligands were utilized as trisodium citrate, lipid mixture 1, and adenosine 5'- triphosphate (ATP). A represents nanoparticle synthesis using 0.5 mM $AgNO_3$ and 5 mM trisodium citrate; while B used 1 mM $AgNO_3$ in presence of 5 mM trisodium citrate. Ag-LM represents nanoparticles synthesised using 1 mM $AgNO_3$ and 100 fold diluted lipid mixture 1. Ag-ATP nanoparticles had 5 mM ATP as surface ligand while the reduced silver core were formed from 1 mM $AgNO_3$.

In brief, an aqueous solution of silver natrate solution was prepared in a clean glass vial and incubated with surface ligands viz. trisodium citrate, lipid mixture 1 and ATP at defined concentrations. The soultion was subjected to heating till boil under vigorous stirring followed by dropwise addition of sodium borohydride until color development.

***Evaluation of silver nanoparticle toxicity using bioluminescent bacteria***

A loopful of bioluminescent bacterial culture was suspended in 5 mL sucrose solution (0.34 M) and vortexed till uniform dissoution of the bacteria. Then, 180 µL of the bacterial suspension was pipetted in the luminometeric cuvette in triplicates. The luminescence decay was observed up to a period of 30 or 60 minutes at a regular interval after the addition of 20 µL synthesised AgNPs viz. A, B, Ag-LM, and Ag-ATP.

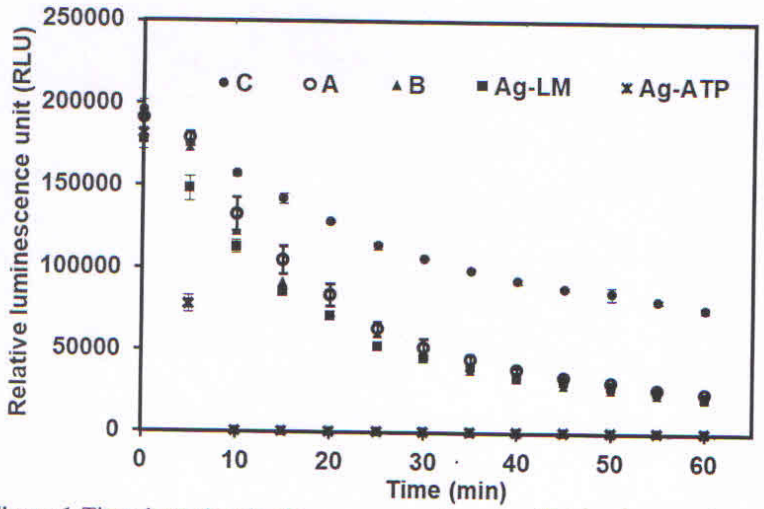


**Figure 1:**Time dependent luminescence measurement of bioluminescent bacteria in response to A, B, Ag-LM and Ag-ATP. C represents luminescence emission in control samples.

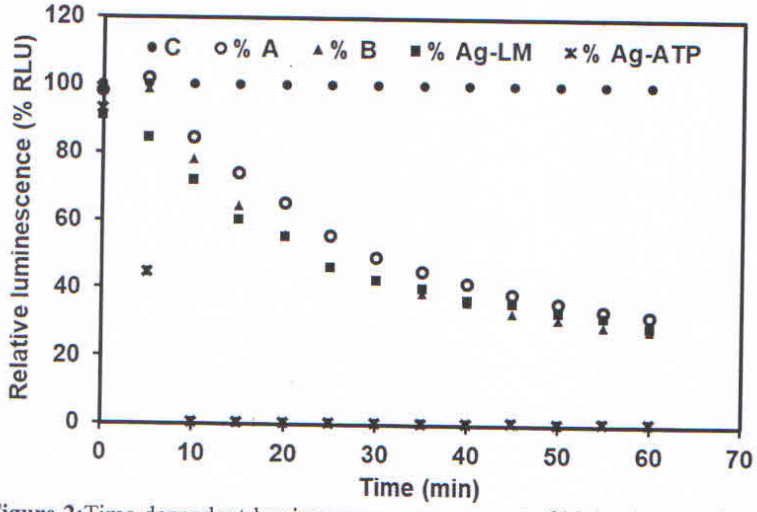


**Figure 2:**Time dependent luminescence measurement of bioluminescent bacteria in response to A, B, Ag-LM and Ag-ATP. Relative luminescence (% RLU) was calculated using the equation $(I/I_0)*100$ where I represents luminescence emission from bioluminescent bacteria post

incubation with silver nanoparticles while $I_0$ represents luminescence response in control samples.

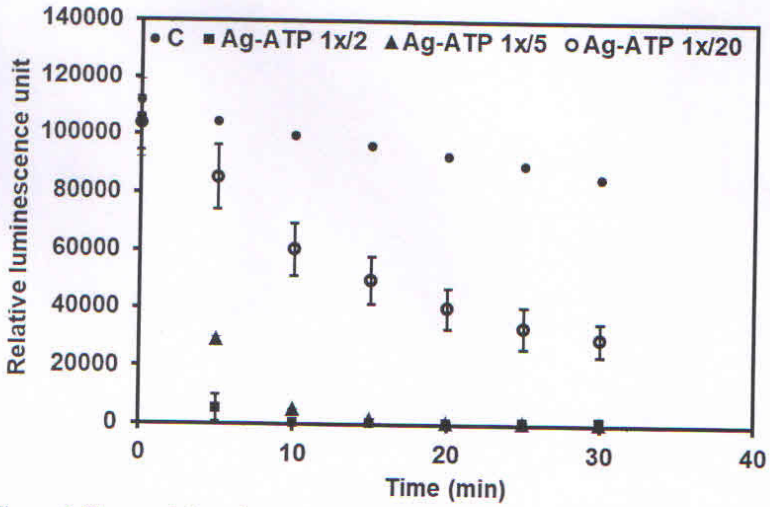


**Figure 3:**Time and doze dependent luminescence measurement of bioluminescent bacteria in response to Ag-ATP at dilution factors of 2, 5 and 20.

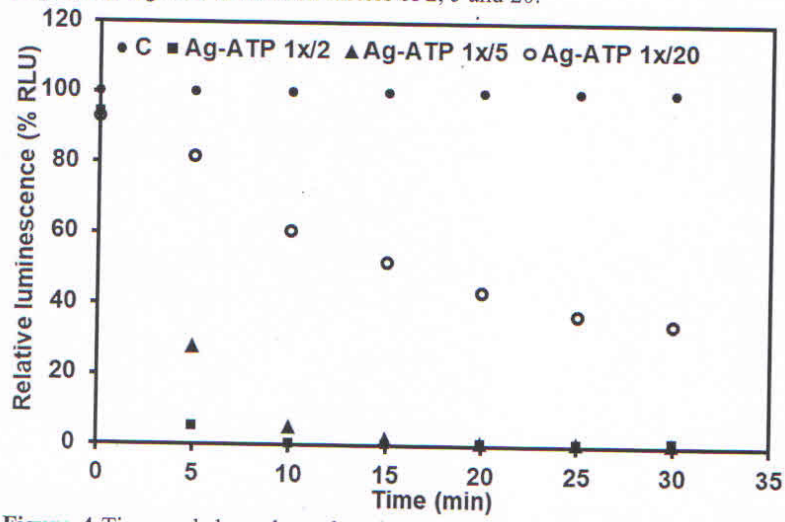


**Figure 4:**Time and doze dependent luminescence measurement of bioluminescent bacteria in response to Ag-ATP at dilution factors of 2, 5 and 20.Relative luminescence (% RLU) was calculated using the equation $(I/I_0)*100$ where I represents luminescence emission from bioluminescent bacteria post incubation with silver nanoparticles while I0 represents luminescence response in control samples.

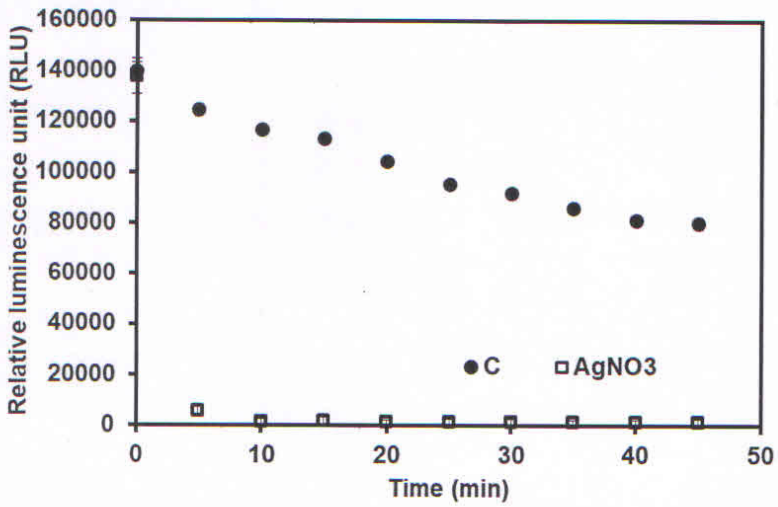


**Figure 4:**Time dependent luminescence measurement of bioluminescent bacteria in response to 0.1 mM $AgNO_3$.

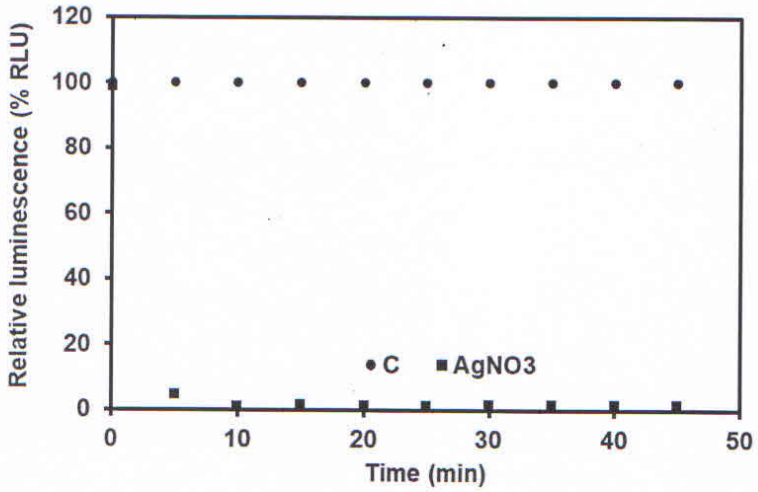


**Figure 4:**Time dependent luminescence measurement of bioluminescent bacteria in response to 0.1 mM $AgNO_3$.Relative luminescence (% RLU) was calculated using the equation $(I/I_0)*100$ where I represents luminescence emission from bioluminescent bacteria post incubation with $AgNO_3$while $I_0$ represents luminescence response in control samples.

The present research investigated the effect of silver nanoparticles (AgNPs) on the bioluminescent bacteria in suspension. Silver nanoparticles were synthesised by varying the

concentration of surface ligands (trisodium citrate, Adenosine 5'-triphosphate) in presence of a strong reducing agent, sodium borohydride ($NaBH_4$). A loopful of bioluminescent bacterial culture was suspended in 5 mL sucrose solution (0.34 M) and vortexed till uniform dissoution of the bacteria. Then, 180 µL of the bacterial suspension was pipetted in the luminometeric cuvette in triplicates. The luminescence decay was observed up to a period of 30 or 60 minutes at a regular interval after the addition of 20 µL synthesised AgNPs.

The results demonstrate that surface ligands dramatically affect the luminescence response from bioluminescent bacteria. ATP capped AgNPs dramatically affected the luminescence in comparison to control. Therefore, further investigation was performed through dilution of ATP capped nanoparticles. The results indicated dose-dependent toxicity. Surface ligands such as trisodium citrate or lipid mixture 1 were also reported to affect luminescence at a lower rate than ATP-capped AgNPs.

Therefore, the ATP plays a pivotal role in inducing toxicity towards bioluminescent bacteria undertaken in the present study. Further, investigation will focus on mechanisms associated with the ATP toxicity.

**Research Publication- Under preparation**

### iii) Proposed utilization of the experience in India

The experience acquired during training led us to a new paradigm shift in the identification of nanoparticles and other chemicals in environment, food and water. Therefore we proposed/prepared the following project based on the techniques/expertise learned during training-

**Bioluminescence based toxicity evaluation remains elusive owing to its simple analytical procedure without the requirement of expensive instrumentation. Preliminary investigation during my training suggests that the capping agent surrounding the reduced silver core ($Ag^0$) plays an important role in regulating AgNPs toxicity towards bacterial cell membrane via unknown mechanisms. It is presumed that membrane transport channels play a crucial role in mediating AgNP induced toxicity. Therefore, further research in this direction is auspicious.**

**The proposed research will explore a) biophysical aspects of AgNP binding to the cellular membrane (e.g. electrostatic, van der Waals, hydrophobic interactions) using bioluminescent bacteria as the model microorganism, b) nature of membrane ion channel inhibition via functional ligands (competitive, non-competitive) and c) AgNP induced cellular toxicity in cancer cell lines expressing P2X receptors.**

**Objectives**

1) Synthesis of AgNPs with a wide variety of functional ligands differing in surface charge, presence of bulky groups, and ion channel inhibitors followed by their morphological, photophysical and chemical characterization using Transmission Electron Microscopy (TEM), UV-Vis spectroscopy, dynamic light scattering measurements (DLS), Fourier-transform infrared spectroscopy (FT-IR), Energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) analysis.

2. Evaluation of dose and time dependent AgNPs toxicity using bioluminescent bacteria in free and immobilized form.
3. Response of ion-channel inhibitors (e.g. Sodium orthovanadate, oligomycin) on luminescence emission characteristics of bioluminescent bacteria.
4. Effect of AgNPs decorated with cationic, anionic and hydrophobic moieties on cellular membrane of bioluminescent bacteria.
5. Validation of toxicity evaluation of AgNPs through luminescence and MIC/MBC assays.
6. AgNP induced cellular toxicity in a cancer cell line expressing P2X receptors.

The expected results of the project:

1. Synthesis of AgNPs with multifunctional ligands, size, shape and zeta potential analysis of AgNPs
2. Determination of minimum inhibitory concentration and minimum bactericidal concentration of AgNPs
3. Ranking of AgNPs toxicity through luminescence and MIC/MBC measurements
4. Establishing the role of AgNPs in inhibiting membrane associated ion-channels
5. Understanding the effect of AgNPs on P2X receptors in cancer cell lines.

ICMR Sanction No.-INDO/FRC/452/S-53/2019-20-IHD

Signature of ICMR-IF
(Dr. Yeshvandra Verma)

Dr. Yeshvandra Verma
Assistant Professor
Department of Toxicology
C. C. S. University, Meerut
250004 India