REPORT

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

1.	Name and designation of ICMR- IF	:	Dr. Joseph SELVIN Associate Professor
2.	Address	:	Department of Microbiology Pondicherry University Puducherry 607 014
3.	Frontline area of research in which training/research was carried out	:	Development of anti-infectives molecules from marine bacteria against *Pseudomonas aeruginosa*
4.	Name & address of Professor and host institute :		Associate Prof **Yang Liang** Nanyang Associate Professor (NTU) Singapore Centre for Environmental Life Sciences Engineering (SCELSE) Nanyang Technological University 60 Nanyang Drive, SBS-01N-27 Singapore 637551
5.	Duration of fellowship	:	3 months (28.02.2018 to 28.05.2018)
6.	Highlights of work conducted	:	

The efforts to identify target specific quorum sensing inhibitor without affecting the growth of the pathogen (biomass) is being considered as an effective anti-virulence strategy to contain pathogenic infections. The anti-virulence methods can be next-generation anti-infectives to overcome the issue of antibiotic resistance. Considering potential virulence inhibitors identified from the marine bacteria, the efficacy was validated with *Pseudomonas aeruginosa* reporter strains (using gfp-green fluorescence protein tagged strains from SCELSE, Nanyang Technological University). In *Pseudomonas aeruginosa*, the main QS (quorum sensing) system are regulated by N-acyl homoserine lactones (AHLs)-based signaling molecules such as las and rhl systems. In the las system, LasI synthase mediates the synthesis of N-(3-oxododecanoyl)-L-homoserinelactone (3-oxo-C12-HSL). The synthesis of AHL is required to induce the expression lasR, a factor induce the virulence cascade genes such as lasB, apr,and toxA. The las system positively regulates rhl system, which mediates the synthesis of N-butanoyl-L-homoserine lactone (C4-HSL) and induce the transcription of rhlR and rhamnolipid synthesis genes rhlAB. The rhl system also involved in the synthesis of rhamnolipid, pyocyanin, and hydrogen cyanide synthase. Therefore marine bacterial products (lipopeptides) were screened using *P. aeruginosa* quorum sensing reporter strain assays targeting the virulence cascades.

i) Technique/expertise acquired : Bioreporter assay based on expression of green fluorescence protein (GFP) *in P. aeruginosa* PAO1 (lasB-gfp, rhlA-gfp, pqsA-gfp) and *P. aeruginosa* (lasB-gfp-Mcherry, rhlA-gfp-Mcherry, pqsA-gfp--Mcherry), Elastase assay, Rhamnolipid quantification assay and Biofilm assay.

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

During the visit, two novel QSIs (quorum sending inhibitors) from marine bacteria was identified through the screening against a well-established QS reporter strain *P. aeruginosa* PAO1-lasB-gfp. Two leads (SW07 and SW-08, active fractions separated from sponge associated marine *Bacillus* spp.) were discovered to inhibit lasB, rhlA, and pqsA expression as judged from bioreporter assay based on expression of green fluorescence protein (gfp) in *P. aeruginosa* PAO1 (lasB-gfp, rhlA-gfp, pqsAgfp). The anti-virulence activity was confirmed in *P. aeruginosa* reporter assay (lasB-gfp-Mcherry, rhlA-gfp-Mcherry, pqsA-gfp--Mcherry), Elastase assay, Rhamnolipid quantification assay and Biofilm assay. The activity was independent of growth, targeting specifically on quorum sensing pathways (quorum sensing inhibitors). Detailed description of the results, data processing, structural elucidation of the compounds, whole genome sequencing of the producer strains and phenotypic assays are under progress.

iii) Proposed utilization of the experience in India :

The anti-infective molecules screened in the quorum sensing reporter assay were developed as a part of ongoing research in Microbial Genomics Lab, Department of Microbiology, Pondicherry University. The assays will be applied in India to screen anti-virulence molecules from the library of bioactive compounds developed from marine bacteria. The proposed utilization of this research training would bring out new leads targeting multi-drug resistant pathogens.

Signature of ICMR-IF

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