DATE: Day____Month____Year 2019

SUMMARY of
2018 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Investigation of Transcriptional Regulators as Potential Drug Targets**
Applicant	**Name**	**Dr. Inoka C. Perera**
	Affiliation	**University of Colombo**
	Present Title	**Senior Lecturer**
Research Collaborator (Host PI)		**Prof. Genji Kurisu**

Summary

Pseudomonas aeruginosa is metabolically versatile gram negative, aerobic, motile, monoflagellated, ubiquitous, opportunistic pathogen predominant in the stratum corneum. They express virulence factors which enable bacteria to thrive in various environments such as soil, water, marine habitats and animal tissues as well as in harsh environments such as hot springs, crude oils as they have very high adaptability. Maintenance of inner and outer membrane is essential for bacteria for active life in harsh environments as it is directly contacting with the environmental substances. Membrane fluidity is tightly regulated by regulating the level of saturated and unsaturated fatty acids in their membrane bilayers. Because imbalance in membrane fluidity is detrimental to the cells. *Pseudomonas aeruginosa* encodes two alkane monoxygenases annotated as AlkB1 and AlkB2 with overlapping substrate range. AlkB1 and AlkB2 facilitate to uptake n-alkanes from C_{10} to C_{24}. n-Alkanes convert into fatty acids via sequential oxidation of terminal methyl group via bacterial metabolism. FadR type regulators have identified in regulation of expression of critical enzymes involved in fatty acid biosynthesis and degradation either as a repressor or activator. PA1526 is a GntR family regulator confine to FadR sub-class in *P. aeruginosa* where divergently transcribed from Alkane-1-monoxygenase 2 (AlkB2) gene. Transcriptional regulator PA1526 in *Pseudomonas aeruginosa* is proposed as a potent drug target to battle infections of *P. aeruginosa* as it regulates an important pathway responsible for the ubiquitous existence of the organism. Three-dimensional (3D) structure of the target protein needs to be determined for structure based drug discovery. Protein structural studies were performed to determine the model of PA 1526. Recombinant PA1526 was purified to near homogeneity to screen crystal formation at different conditions. However, Crystallization of PA1526 was not successful. Circular dichorism (CD) spectroscopy revealed PA 1526 has high number of α helixes and melting temperature of PA1526 is 40 ℃. Transcriptional regulation is a fundamental biological process crucial for Mycobacterium tuberculosis for its survival in macrophages upon various stress conditions including nutrient deprivation, hypoxia, acidic pH, reactive oxygen species, reactive nitrogen species and antibiotics. *Mycobacterium tuberculosis* Rv0792 regulator protein, considered to be responds to oxidative stress or in an important cell cycle pathway or antibiotic synthesis pathway.

As the last year work summary, the constructed pET 28a with Rv0792 gene was transformed into *E. coli* BL21(DE3) for overexpression with IPTG. The expressed Rv0792 protein was purified using Ni-NTA

chromatography and further purified using size exclusion chromatography following TEV protease cleavage of His tag. A final concentration of 5 mg/ml of protein was obtained with 90% homogeneity. Crystal screening was carried out for this concentration using screening kits, Crystal screen I & II, Wizard I, II, III & IV and PEG/Ion I & II. The crystals observed under different conditions were confirmed as salt crystals. CD spectrum and DSC analysis was unsuccessful due to protein aggregation upon higher concentrations. DLS analysis was carried out for protein with different buffer conditions of different pH in order to determine the particle size upon precipitation and to analyze the solubility of protein. The lowest aggregation was observed with higher homogeneity, for MES (pH 5.5) buffer.

***Deadline: May 17, 2019**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
***We accept only PDF file. Please file it after converting WORD to PDF.**
***Please describe this summary within 1 sheet. Please DON'T add some sheets.**
***This summary will be published on the web.**