

Report on Visit to Prof. Philip Poole Lab (16th July -14th Sept. 2018)

Hello All,

I am Kedharnath Reddy Pengani, working as a research fellow under guidance of Dr. K. Annapurna, Head, Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India. My lab is one of the participating labs in the India-UK Nitrogen Fixation Centre. The focus of our lab is “Harnessing the Diversity of diazotrophs in two contrasting rice genotypes and their potential for plant growth promotion.” The samples were collected from these genotypes grown under three levels of Nitrogen, 0%, 50% and 100%. Using cultivable and uncultivable methods, we are trying to analyze the microbial community structure and the abundance of diazotrophic community.

My visit to Prof Phil Pooles’ laboratory in Oxford University, Department of Plant Sciences was to learn the tools and techniques for analyzing the metagenomic data. My visit was for two months (16th July -14th Sept. 2018).

Metagenome Analysis and Visualization

I carried out Metagenome raw data pipeline development and execution, microbial diversity analysis and visualization, and Root Exudates identification, in Prof. Poole lab.

I have done the metagenome analysis and visualization under guidance of Dr. Andrzej Tkacz. Andrzej introduced me to Linux terminal commands, and pipeline for metagenome raw reads processing (USEARCH 10 pipeline, Megan Visualization, XLstat basics), and data visualization using Primer7. I learnt the use of command line user interface, like opening terminal, installing softwares in linux, unzipping files, editing lines in a file, etc., and processing of multiple sequence files at a time using commands in Usearch10 pipeline. Pipeline mainly consists of quality check of Fastq files from sequencing company using Usearch Fastx_info command, basic edit commands like sed, grep and awk, merging the reads using fastq_merge_pairs by Usearch10 and Flash (fast length adjustments of short reads) commands, writing reverse compliment to reverse reads using fastx_reverse_compliment command, merging the reads using cat command, deleting singletons, making OTU table using Usearch10, and annotating sequence reads using NCBI-BLAST using SILVA database in machine itself.

Data visualization of processed reeds and annotated reeds, in the form of α -diversity (OTU Richness, Shannon diversity indices) β -diversity (nMDS, PCO) and bacterial abundance using MS Excel sheets bar graphs was carried out.

Root Exudates Identification using Biosensors

Dr. Allison East and Dr. Beatriz Jorrin helped me to identify root exudate components using biosensors (different strains for different root exudates like Amino acids, Sugars and Organic Acids), strain database is listed in IUNFC website. Using UMS agar plates, rice root exudates were placed in middle of the plate and 16 strains (Biosensors) were streaked on these plates, after incubation at 28°C for 48hrs and 72hrs, plates were checked in NightOwl instrument by Dr. Beatriz Jorrin.

Experience in Oxford

It is a wonderful lab with awesome people with cutting edge research. I still miss working with Dr. Andrzej. He helped me throughout my visit, patiently clearing all my doubts. I am thankful to Dr. Allison and Dr. Beatriz Jorrin for their help with root exudates experiment and Dr. Vinoy Ramachadran for support in every way.

And finally I am very thankful to Dr. K. Annapurna and Prof. Phillip Poole for this wonderful opportunity to enhance my research capabilities and skills in metagenome analysis.

Thank you Poole Lab,