

Hello everyone!

I am Anirban Basu from Prof. Appa Rao Podile's lab at University of Hyderabad. As one of the participating Indian labs in the IUNFC project, our lab is involved in optimizing pigeon pea and rhizobial inoculant combinations for improving pigeon pea yields in the Alfisols of south India.

I got the golden opportunity to visit two premier UK labs for three months last summer (June-August 2019). I visited the James Lab, James Hutton Institute (JHI), Dundee during 6<sup>th</sup>-16<sup>th</sup> June and 1<sup>st</sup>-31<sup>st</sup> July 2019 and the Poole Lab, University of Oxford (UoO) during 16<sup>th</sup>-30<sup>th</sup> June and 1<sup>st</sup>-31<sup>st</sup> August 2019. Working in both the labs was an enriching experience for me where I was introduced to and given hands-on training on cutting edge molecular biology and microscopy techniques to study rhizobial genetics and anatomy.

My project involved:

1. **Transmission Electron Microscopy of pigeon pea nodules (JHI):** I was given hands-on training on sample preparation and ultramicrotome sectioning of resin-embedded pigeon pea nodules infected by rhizobial strains from University of Hyderabad (plants grown and harvested at JHI) for light microscopy and TEM studies of nodule anatomy and rhizobial colonization pattern.
2. **Immunogold labelling for TEM (JHI):** Immunogold labelling was done to study *nifH* gene expression in betarhizobia (*Paraburkholderia* sp.) infected pigeon pea nodules using betarhizobial NifH-specific antibody.
3. **Golden Gate Cloning and transformation (UoO):** I was introduced to the basics of Golden Gate cloning techniques and modular assembly of Golden Gate-compatible vectors. Golden Gate reactions were set up to express GusA and CelB markers from Golden Gate vector modules. The Golden Gate cloning cassettes were transformed into competent *E. coli* ( $\lambda$ pir) cells, and the transformants were selected on antibiotic supplemented growth media. Colony PCR was done to confirm successful transformation.
4. **Labelling of *Rlv* 3841 via conjugation-mediated plasmid transfer (UoO):** *Rhizobium leguminosarum* 3841 (wild type) was conjugated with pOPS (non-self-transmissible) plasmids integrated with GFP module having different promoters (J231XX) and pRK2013 (self-transmissible helper) plasmid. The GFP fluorescence intensity of transconjugants (*Rlv* 3841 mutants) was measured in a plate reader.
5. **Labelling of *Mesorhizobium* spp. via conjugation mediated plasmid transfer (UoO):** *Mesorhizobium* spp. (*M. australicum*; *M. cicero*; *M. opportunistum*) were labelled with GusA and CelB. For this, Tn7 transposon-based plasmid pTNS3 (for chromosomal integration) was used for conjugation along with pRK2013 (self-transmissible helper) plasmid.

My visit to both the UK labs, albeit for a short duration, was a successful one. I wholeheartedly thank Prof. Philip Poole and Dr. Euan James for kindly accommodating me into their respective labs. Travelling by train to and fro between the two labs was a mesmerising experience to enjoy the scenic beauty of England and Scotland.

I am grateful to Dr. Euan James and Dr. Marta Maluk, for their constant guidance and motivation, both professionally and personally. Dr. Euan has been very supportive and made me feel at home during my stay at Dundee. Many a time, he went out of his way to help me out, including picking me up and dropping me off to the station twice! He has helped me to acquire an overview of the advanced microscopic techniques right from sample preparation to handling an ultramicrotome.

Dr. Beatriz Jorin had helped me throughout my stay in Oxford right from planning the experiments to executing them. She was always available to help me and clear my doubts. Of the many things that I learnt from her, proper planning and time management have influenced me a lot. I am also thankful to Dr. Alison East and Dr. Vinoy Ramachandran for their all-round support and kind hospitality. During my stay at Oxford, I got the privilege to attend the Oxford Congress on Plant Sciences on 15<sup>th</sup> August 2019. It was a one-day departmental meeting where the research scholars delivered excellent research talks, followed by a barbeque session on the lawns.

Thank you, everyone, for this amazing learning experience and sweet memories that I will cherish forever!

