Armand-Frappier Outstanding Student Award Recipient:

Dr. George diCenzo, McMaster University, Hamilton, ON

Dr. George diCenzo completed his B.Sc. in Molecular Biology and Genetics at McMaster University in 2012. He has just completed his PhD training in the lab of Dr. Turlough Finan at McMaster University. He is now beginning a postdoctoral fellowship in the group of Dr. Alessio Mengoni at the University of Florence in Italy. His research focuses on understanding the genetics and metabolism of the bacterium *Sinorhizobium meliloti*, a model species for the study N2-fixing plant symbionts. His work has involved the use individual genes, and *in silico* genome-scale metabolic network reconstruction. Using this multi-faceted approach, his work has contributed to the understanding of the evolution and role of the complex *S. meliloti* genome structure, characterization of genes important for an effective symbiotic relationship or for competitive fitness as a free-living organism, and the development of wet-lab and *in silico* genomic resources for further characterization of these processes. In the long term, he hopes to develop novel strategies for engineering of rhizobium – legume symbioses, as well as for producing synthetic N2-fixing symbioses with non-legume plants.

Experimental and *in silico* guided approaches to engineering the rhizobium – legume symbiosis.

George DICENZO, McMaster University, Hamilton, ON

Rhizobia are bacterial species capable of fixing atmospheric nitrogen gas to ammonium in symbiosis with a plant host. Symbiotic nitrogen fixation (SNF) is an agriculturally and ecologically important process, but unfortunately we are unable to harness the full power of this biological process in agricultural systems. Our group studies Sinorhizobiummeliloti, a symbiont of legumes such as alfalfa, whose genome consists of a 3.7 Mb chromosome as well as the secondary 1.7 Mb pSymB and 1.4 Mb pSymA replicons. In this talk, I will describe our development of experimental and in silico resources for the study and engineering of SNF. We have developed a genetic background strain for identification of the core genetic chassis required for SNF. This involved the cloning and stable re-introduction of a 69-kilobase region into the S. meliloti chromosome that translocated to the pSymB replicon in a recent ancestor, followed by the reduction of the S. meliloti genome by 45% through the complete removal of pSymA and pSymB. In order to localize all single-copy genes essential for SNF on pSymA/pSymB, we produced a library of large-scale (50-800 kilobases) defined deletion on these replicons, and screened these deletion mutants for symbiotic phenotypes with alfalfa. We found that most regions of pSymA could be removed with no apparent effect on the rate of SNF, and we have constructed deletions of greater than 50% of each replicon without abolishing symbiotic abilities. Indeed, less than 12% of each replicon was absolutely essential for SNF with alfalfa, and these regions are currently serving as our initial target of a minimal symbiotic genome. Through the above described screen and follow-up genetic studies, a previously uncharacterized gene (smb20752) encoding a putative 3-hydroxyisobutyryl-CoA hydrolase, was identified as required for efficient SNF. Strikingly, deletion of the orthologous gene in Sinorhizobiumfredii NGR234 completely abolished SNF. Moreover, the screen described above led us to determine that the BacA protein of S. meliloti is specialized to support symbiosis with plants of the genus Medicago, and that the BacA proteins of the closely related species (S. fredii or Rhizobium leguminosarum) cannot fulfil this function. Complementing this experimental approach, we performed a S. meliloti genome-scale metabolic network reconstruction for modelling metabolism and metabolic consequences of gene mutations in free-living and symbiotic cells. The metabolic reconstruction accounts for 1575 genes, it is constantly being improved through manual curation, and can serve as a platform for the coherent integration of systems-level datasets. Through growth simulations with flux balance analysis, we have examined the genetics involved in high fitness colonization of bulk soil, the rhizosphere, and SNF. Ongoing work involves using in silico simulations to identify targets for biotechnological manipulation to improve the symbiosis.