Ph.D. projects in progress

1.

Mentor: Gabriella Endre

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. Student: Szilárd Kovács

Title of the research topic: Genetic dissection of legume-rhizobia symbiosis via *Tnt1*-insertion mutagenesis in *Medicago truncatula*

Description of the research topic: Legumes are special among flowering plants in their ability to establish symbiotic associations with nitrogen-fixing bacteria collectively known as rhizobia. Revealing and understanding the functions of genes and proteins involved in the legume-rhizobia symbiosis have a great importance not just in the basic but also in the applied research. A very efficient way of identifying important genes in model plants is the forward and reverse genetic analyses of mutants. The use of tagged mutant collections has already proved to be successful in revealing plant genes that function in the nitrogen-fixing symbiosis. However, it is likely that not all plant symbiotic genes have been identified yet. In this work we use the Tnt1 insertion mutant collection of the model legume M. truncatula cv. Jemalong. A large scale symbiotic screen was done using this mutant collection and those lines were selected in which individuals with impaired symbiotic phenotype appeared. We focus on those mutants that indicated defects in different steps of the symbiotic process, thus 24 lines were chosen for back-cross and further genetic analyses. From these lines segregating populations were produced for 11 mutants. In the meantime, FST sequences belonging to these lines were also carefully analyzed to use candidate gene approach. Thorough phenotype characterization and genotype determination of the candidate genes resulted in the identification of the mutated gene responsible for the symbiotic phenotype in two different mutant lines. One of them is a novel gene playing role in the early bacterial invasion of the nodule and the other is a new allele of the recently cloned NAD1. The characterization of these two genes and their protein products reveal their roles during the early symbiotic stages.

2.

Mentors: Attila Kereszt, Éva Kondorosi

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. Student: Ting Wang

Title of the research topic: Rhizobial proteins involved in the incompatible interactions of Sinorhizobium meliloti strain Rm41 with different ecotypes of Medicago truncatula

Description of the research topic: The symbiosis between rhizobia and legume plants is considered to be a mutualistic interaction, however, more and more evidence has appeared that the symbiotic partners has constantly evolved mechanism to maximize their own benefit from the partnership. Plants may produce a plethora of peptides in the invaded cells to direct the terminal differentiation of the bacteria resulting in higher nitrogen-fixation efficiency. Bacteria may evolve accessory functions which increase their ability to cheat the plants and to form a larger population of undifferentiated cells within the symbiotic organ that will be released into the environment after the symbiosis ceases to function. In most cases, these genes do not significantly inhibit nitrogen-fixation, however, they may cause symbiotic incompatibility in interactions with a partner which carries a certain gene or gene variant. Bacterial and plant genes, gene variants causing symbiotic incompatibility between Sinorhizobium meliloti strain Rm41 and Medicago truncatula ecotypes Jemalong and F83005 are being identified and characterized.

3.

Mentors: Attila Kereszt, Éva Kondorosi

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. Student: Senlei Zhang

Title of the research topic: Developing tools for the functional analysis of NCR peptides in Medicago truncatula

Description of the research topic: During the Ph.D. project, two new reporters systems have been developed that are based on the accumulation of the purple colored anthocyanins and the complementation of the non-nodulating nsp2 mutant, respectively. In the anthocyanin system, the over-expression or vascular tissue specific expression of the MtLAP1 gene in transgenic hairy roots and/or nodules results in purple coloration from anthocyanin accumulation and, thus, the transgenic tissues can be identified by naked eyes. In the case of the NSP2 reporter, which codes for an transcription factor essential for the initiation of nodule development, hairy root transformation is performed on the Nod- nsp2 mutant plants. As a result, all the formed nodules are transgenic and their as well as the plants' phenotype depends on the nodule genotype, for example, after CRISPR/CAS9 gene editing. These two systems can significantly reduce the labor in transgenic root detection. To look for the supposed conserved transcription regulators of the NCR169 gene coding for a nodule-specific cystein-rich peptide, which is essential for the formation of a functional nodule, the DNA pull-down, Y1H screening and EMSA techniques were combined and materials and cDNA libraries from both Medicago and soybean nodules were used.

4.

Mentor: Attila Kereszt

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. Student: Benedikta Balla

Title of the research topic: Role of rhizobial proteins in the incompatible interaction between Sinorhizobium meliloti strain GR4 and Medicago truncatula cv. Jemalong

Description of the research topic: The symbiosis between rhizobia and legume plants is considered to be a mutualistic interaction, however, more and more evidence has appeared that the symbiotic partners has constantly evolved mechanism to maximize their own benefit from the partnership. Plants may produce a plethora of peptides in the invaded cells to direct the terminal differentiation of the bacteria resulting in higher nitrogen-fixation efficiency. Bacteria may evolve accessory functions which increase their ability to cheat the plants and to form a larger population of undifferentiated cells within the symbiotic organ that will be released into the environment after the symbiosis ceases to function. In most cases, these genes do not significantly inhibit nitrogen-fixation, however, they may cause symbiotic incompatibility in interactions with a partner which carries a certain gene or gene variant. Bacterial and plant genes, gene variants causing symbiotic incompatibility between Sinorhizobium meliloti strain Rm41 and Medicago truncatula ecotype Jemalong are being identified and characterized. In addition, to study the interaction of the identified proteins with other rhizobial protein a bacterial two-hybrid library carrying all predicted coding sequences of Sinorhizobium meliloti strain 1021 is being constructed and tested.

5.

Mentor: Attila Kereszt

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. Sutdent: Csaba Gellért

Title of the research topic: New synthetic biology methods for nitrogen-fixing bacteria to identify and study genes required for the infection and invasion of symbiotic root nodule

Description of the research topic: The aim of the Ph.D. project is the development and adaptation of synthetic biology tools for rhizobia that can be used for the investigation of the function of bacterial genes required for the growth of infection threads that take bacteria into the cells of the developing symbiotic nodule, a de novo formed organ to host symbionts. The tool kit will allow to study the role of those genes during infection thread growth that are essential for the infection thread initiation, i.e. their mutations prevent the initiation step. In addition, we will investigate the transcriptomic responses wild type or receptor mutant plants towards bacteria that, for example, cannot maintain infection thread growth because they produce no or structurally modified surface polyasaccharides.

6.

Mentors: Gabriella Endre, Éva Kondorosi

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. student: Anas M. Al Bouni

Title of the research topic: Characterization of plant-derived NCR peptides and their effect on microbes

Description of the research topic: Rhizobium-legume symbiosis represents a fascinating system of chemical ecology using a wide range of chemical and peptide signals for consecutive communication events between the host plant and its Rhizobium

partner. In the soil, plant signal molecules selectively target the Rhizobium partner out of billions of microbes and induce in the bacteria the synthesis of host-specific Nod factors required for root nodule organogenesis and infection of plant cells with bacteria. Inside the plant cells the bacteria differentiate into nitrogen fixing bacteroids. In legumes like alfalfa or pea, the bacteria undergo a remarkable and irreversible differentiation process manifested by elongation and branching of bacteria in association with the amplification of their genome, loss of their cell division ability and alteration of their membrane structure. The successive steps of this terminal bacteroid differentiation are plant directed and mediated by symbiotic nodule-specific cysteine-rich NCR and nodule-specific glycine-rich GRP peptides. In Medicago species more than 700 NCR peptides and 25 GRP peptides are expressed at different developmental stages of the symbiotic cells and provoke sequential maturation of the endosymbiont. Many of these plant peptides act via multi-hit mechanisms by interacting with the bacterial membranes and binding to diverse bacterial targets in the cytosol. Many cationic NCR peptides also have broad-spectrum antimicrobial activity effectively killing various human and plant pathogen bacteria and fungi including antibiotic resistant strains without toxicity on human cells. Selected peptides with strong antimicrobial activity on various pathogenic bacteria and fungi are currently under investigation as lead molecules for future antibiotics. The Ph.D. project will focus on a few selected and so far uncharacterized NCR peptides that show high expression at specific stages of symbiotic cell differentiation. Knock out mutants will be generated and symbiotic phenotypes of gene inactivation will be studied including transmission and scanning electron microscopy. Targets of the peptides will be identified with pull down experiments coupled to mass spectrometry. Peptides will be screened for antimicrobial activities as well and the mode of action of the active peptides will be investigated.

7.

Mentor: Éva Kondorosi

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. student: Rui Dániel Lima

Title of the research topic: The role of NCR peptides in symbiosis and in vitro

Description of the research topic: In *Medicago truncatula-Sinorhizobium meliloti / medicae* symbiosis, nitrogen-fixing bacteria are the result of an extraordinary differentiation process. This process is driven by hundreds of plant peptides, of which about 700 peptides belong to the nodule-specific cysteine-rich NCR peptide family. These secreted peptides are produced only in symbiotic plant cells where they interact with the bacteria. The peptides abolish the cell division ability of bacteria and turn them into huge polyplod cells. The signal peptide of peptides is relatively conserved, while the sequence of mature peptides can be highly variable and can be a source of an extremely wide variety of biological activities. One of the aims of the doctoral dissertation is to understand the function of some selected NCR peptides and to explore what may be the reason for the conservation of the signal peptide.

8.

Mentor: Péter Kaló

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. student: Alexandra Pál

Title of the research topic: Identification of plant genes involved in symbiotic nitrogen fixation.

Description of the research topic: Legumes compose the third largest family of flowering plants. Medicago truncatula and other leguminous plants are able to establish nitrogen-fixing symbiotic associations with soil bacteria belonging to the genus rhizobia. The legume-rhizobial symbiosis accounts for a significant proportion of biological nitrogen fixation worldwide. Symbiotic nitrogen fixation takes place in specialized organs on the root, termed nodules. The aim of our research is to study the molecular steps of the induction, invasion and function of the symbiotic nodule to better understand the molecular basis of symbiotic nitrogen fixation. We use plant symbiotic mutants defective in induction of nodule development ((Nod- mutants) or normal functioning of the symbiotic nodule (Fix- mutants) to identify genes required for symbiotic nitrogen fixation. The PhD student's project is to characterize the symbiotic phenotype of the mutants, clone the impaired genes and perform their detailed functional analysis to identify the role of the symbiotic genes in the rhizobium-legume symbiotic interaction.

9.

Mentor: Péter Kaló

Doctoral School: University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology

Ph.D. student: Bilguun Tsenddorj

Title of the research topic: Identification of plant genes involved in symbiotic nitrogen fixation

Description of the research topic: Legumes compose the third largest family of flowering plants. Medicago truncatula and other leguminous plants are able to establish nitrogen-fixing symbiotic associations with soil bacteria belonging to the genus rhizobia. The legume-rhizobial symbiosis accounts for a significant proportion of biological nitrogen fixation worldwide. Symbiotic nitrogen fixation takes place in specialized organs on the root, termed nodules. The aim of our research is to study the molecular steps of the induction, invasion and function of the symbiotic nodule to better understand the molecular basis of symbiotic nitrogen fixation. We use plant symbiotic mutants defective in induction of nodule development ((Nod- mutants) or normal functioning of the symbiotic nodule (Fix- mutants) to identify genes required for symbiotic nitrogen fixation. The PhD student's project is to characterize the symbiotic phenotype of the mutants, clone the impaired genes and perform their detailed functional analysis to identify the role of the symbiotic genes in the rhizobium-legume symbiotic interaction.