Intimate alliances: Plants and their microbial symbionts (TTPB19) – Teaching Guide

Overview – This lecture describes two very intimate symbiotic mutualisms: one is that which occurs between bacteria (rhizobia or *Frankia*) and their plant hosts resulting in the production of nitrogen-fixing nodules. The other is the interaction between two different types of mycorrhizal fungi and their plant hosts resulting in enhanced nutrient uptake. Successful formation of these mutualistic symbioses is a complex process that requires signaling and recognition, morphological and physiological responses, and biochemical contributions from both the plant and microsymbiont.

Learning objectives

_By the end of this lecture the student should be able to:_
- Identify the major forms of symbiosis between plants and microsymbionts and the types of plants, bacteria and fungi involved
- Evaluate the economic and ecological importance of each type of symbiosis
- Indicate which symbiosis is thought to be the most ancient and which is the most prevalent
- Evaluate the roles of industrial and biological nitrogen fixation in natural and agricultural ecosystems
- Describe the signals exchanged between legumes and most rhizobia in the establishment of symbiosis
- State three differences between the process of nodulation by rhizobia and *Frankia*
- Identify what is meant by the common symbiosis pathway and the evidence for it
- Define two ways that plants maintain control over their symbiotic partners
- Compare and contrast arbuscular mycorrhizal symbiosis and ectomycorrhizal symbiosis
- Analyze the costs and benefits of symbiosis to both partners

Study / exam questions (understanding and comprehension)
- What is a rhizobium?
- True or False – all rhizobia are closely related
- True or False – most rhizobia can fix nitrogen as free-living organisms
- Nitrogen makes up 78% of the earth’s atmosphere, but many plants are nitrogen-limited for growth. Why?
- What is the name of the enzyme responsible for biological nitrogen fixation?
- What are the names of the two major groups of plants that fix nitrogen?
- What are three reasons humans cultivate legumes?
- Rhizobia respond to what type plant-produced compounds?
- Many rhizobia produce what compounds to initiate the process of nodulation?
- What are two differences between nitrogen fixation in symbiotic nodules and nitrogen fixation in free-living diazotrophs?
- Describe the steps that occur in the recognition, infection and nodulation process
- What is a symbiosome?
- What is endoreduplication and how does it affect bacteroid viability and ability to fix nitrogen?
- What is leghemoglobin and how does it enhance symbiotic nitrogen fixation?
- Why is it so important for plants to exert tight controls over bacterial infection, nodulation and nitrogen fixation?
- What evidence supports the idea that rhizobia “hijacked” the AM symbiosis pathway?
What are three differences between AM fungi and EM fungi?
Why do we have genomic sequence data from EM but not AM fungi?

Discussion questions (engagement and connections)
- What is “horizontal gene transfer”? What evidence supports the assumption that nod and nif genes have spread by horizontal gene transfer? What other bacterial traits are spreading through this mechanism?
- SYMBIOSIS RECEPTOR-LIKE KINASE is required for symbiosis, but no ligand is identified. Design a set of experiments to investigate the function of the SYRMK protein.
- Once inside a plant cell, what if anything prevents rhizobia from becoming pathogens?
- What are calcium oscillations and how do they carry information? How do proteins decode calcium signals? Use the internet to find another signal transduced by calcium oscillations. Are any of the components of this other system present in the symbiosis system?
- Genetic studies indicate that Myc-factor and Nod-factor signals are both transduced through the SYM genes. How do you think the plant cells respond to the right upstream signal (e.g. prepare nodules in response to Nod factor)? Design an experiment to investigate this question.
- Plant flavonoids induce the transcription of nod genes in rhizobia by binding to and activating the NodD transcription factor. Describe two different experimental approaches to investigate whether plant strigolactones have a similar effect in arbuscular mycorrhizal fungi.
- Our understanding of the perception of and response to plant signals by mycorrhizal fungi is less complete than those of rhizobia. What is one practical reason for this difference in our depth of understanding of these organisms?
- Cyanobacteria can live within plant tissues, but the transfer of fixed nitrogen to the plant is poor. By contrast, rhizobia export fixed nitrogen to the plant with high efficiency. What is the basis for this difference in efficiency?
- Extending efficient nitrogen-fixing symbiosis to other crop plants would have a significant benefit. How feasible is it? How would you go about this process?
- This lecture describes plant symbioses with rhizobia, Frankia, arbuscular mycorrhizal fungi and ectomycorrhizal fungi. One of these is somewhat different from the other three. Which type of microsymbiont is the odd-one-out, and why?
- Op den Camp et al (2011) (Science 331: 909-912) found that in Parasponia the same LysM-type receptor is involved in perception of signals from AM fungi and rhizobia. What do their results suggest in terms of the evolution of the rhizobial symbiosis? (A nice summary of their article is in the same issue: Science 331: 865-866).
- The AM fungi, the Glomeromycota, are unusual and challenging to study. Besides being obligate symbionts, they are asexual, coenocytic, and have significant nuclear genetic diversity within an individual. Whether you prefer a genetic, genomic or evolutionary point of view, learning more about these organisms and the challenges they present to scientists requires a little extra effort. Are you ready to be challenged? Here are a couple of accessible references to get you started:
Lecture synopsis
Introduction (1 - 3)
Plants form intimate symbioses with nitrogen-fixing bacteria and mycorrhizal fungi. Some plants form bacteroid-filled nodules that fix nitrogen from which to assimilate fixed nitrogen. Most plants associate with mycorrhizal fungi through which their assimilation of water and nutrients is enhanced.

ROOT NODULE SYMBIOSIS (4 – 84)
Most plants that form nitrogen-fixing nodules are legumes. Their partners are diverse bacteria collectively called rhizobia. Unrelated Frankia bacteria nodulate a diverse group of plants called actinorhizal plants. The symbiosis is mutually beneficial, with the plant providing the bacteria with fixed carbon, and the bacteria providing the plant with fixed nitrogen.

Biological nitrogen fixation (4 – 18)
Nitrogen gas is abundant, but unavailable to most organisms. Nitrogen fixation involves formation of ammonium from N₂, which requires a high input of energy. Industrial nitrogen fixation produces agricultural fertilizers at the expense of fossil fuels. Biological nitrogen fixation uses the enzyme nitrogenase and ATP to fix nitrogen. Only some prokaryotes called diazotrophs can do this. Some of these diazotrophs form symbiotic organs (nodules) with plants that supply the plants with nitrogen. The most well understood of these interactions is the one between legumes and rhizobia.

Legumes and rhizobia – distribution and significance (19 – 21)
There are 20,000 species of legumes, many of which form nodules in association with rhizobia. Rhizobia are a very diverse group of bacteria defined functionally by their ability to nodulate legumes. The nod and nif genes carried by rhizobia are thought to have been transferred horizontally, because these genes are tightly linked on symbiotic islands or plasmids, and because they are more closely related to other nod and nif genes than would be expected based on the phylogenetic relationships of the bacteria they are found in.

Legumes and rhizobia – communication and signaling (22 – 42)
Communication between bacteria and host – flavonoids and Nod factors (24 – 32)
Rhizobia recognize flavonoids extruded from plant roots, and respond to them by producing Nod factors (lipo-chitooligosaccharides). Plants respond to Nod factors through Nod-factor receptors. Some rhizobia are able to infect plants independently of Nod factors.
The common SYM pathway (33 – 42)
Downstream of Nod-factor perception there is a signal transduction pathway that leads to nodule formation. At least seven genes are necessary both for rhizobial symbiosis and for symbiosis with arbuscular mycorrhizal fungi; these genes are called the common symbiosis genes or SYM genes. These genes outline a signal transduction pathway from plasma membrane to nucleus.
Calcium signaling (36 – 41)
Microbial symbionts including rhizobia, arbuscular mycorrhizal (AM) fungi and Frankia elicit a calcium-spiking response in the host root hair. The calcium oscillations are decoded by a calcium and calmodulin dependent protein kinase (CCaMK) which is central to the plant’s responses. A CCaMK knock-out does not respond engage in symbiosis, and a gain-of-
function mutant forms spontaneous nodules. CCaMK in turn regulates the activity of symbiosis-associated transcription factors.

**Legumes and rhizobia – bacterial entry and nodule development (43 - 57)**

**Bacterial entry, infection thread formation and bacteroids differentiation (44 – 50)**

Bacterial infection is the process by which bacteria move into the developing nodule. Some bacteria move through cracks in the epidermal cell and others through root hairs via infection threads. Within the root cortex, the bacteria move into the plant cells as membrane bound organelles called symbiosomes. They also differentiate into bacteroids capable of fixing nitrogen.

**Nodule morphology and development (51 – 57)**

Nodules are quite variable. Some are determinate and some are indeterminate. Hormone particularly auxin and cytokinin have a role in nodule development. *Sesbania rostrata* can form nodules on its stem. Through genetic studies of *Sesbania* and other legumes it is possible to distinguish the requirements for bacteria entry from those of nodule development, leading to the model that they are largely distinct parallel processes.

**Legumes and rhizobia – coordination of the symbiosis (58 – 77)**

**Symbiotic nitrogen fixation requires teamwork (58 – 69)**

The differentiated bacteroid shuts off most of its metabolic pathways and becomes dependent on the host. The plant provides the bacteroids with organic carbon compounds which are used to produce the ATP needed by nitrogenase. Nitrogenase is a bacterial enzyme. However, a cofactor, homocitrate, is provided by the host (free-living diazotrophs make their own homocitrate). The rhizobia also shut off glutamine synthase, the enzyme that assimilates ammonium into amino acids, and they become dependent upon their host for organic nitrogen and amino acids. In many ways the symbiosomes resemble a nitrogen fixing organelle.

**Host control of nodulation (70 – 76)**

The plant controls nodulation and nitrogen fixation at every step. Nodule formation is suppressed in plants with ample nitrogen, and the regulation of nodule number is controlled. This is called autoregulation of nodulation (AON) and plants deficient in AON can be hypernodulating. Nodules that do not provide the host with fixed nitrogen are sanctioned.

**Root nodule symbiosis – beyond the legumes (78 – 83)**

**Parasponia and rhizobia (78 – 79)**

Some rhizobia can nodulate a non-legume, probably through an independent evolutionary event that provides an interesting system in which to study the genetic requirements for nodulation.

**Actinorhizal plants and Frankia (80 – 83)**

*Frankia* is a single genus of bacteria that can nodulate approximately 200 species of plants, mostly trees. *Frankia* are slow-growing filamentous bacteria that are difficult to cultivate, but the genomic sequences of a few species are completed. *Frankia* do not make Nod factors and it is not known how they communicate with their hosts. Actinorhizal nodules are modified lateral roots derived from the pericycle, and quite different from the nodules that form in legumes.
PLANTS AND MYCORRHIZAL FUNGI (85 - 109)
Mycorrhizal (literally means fungus root) fungi extend the ability of the plant to forage for nutrients in the soil. In most cases the fungus takes organic carbon from the plant, in exchange for greatly enhanced uptake of nutrients especially phosphate but also nitrogen, and water.

Arbuscular mycorrhizal symbiosis – distribution (89 – 91)
The fungal partners in arbuscular mycorrhizal (AM) symbiosis are the Glomerales, a small monophyletic group that interacts with about 80% of terrestrial plants. There is evidence that this interaction has persisted for ~ 460 million years, and it probably originates from around the time that the earliest plants moved onto land. AM symbiosis and functional SYM genes are found across the plant family tree.

Arbuscular mycorrhizal symbiosis – signaling (92 – 100)
AM respond to strigolactones, plant-produced hormones. The fungi produce signals called Myc factors that trigger responses in plants, including calcium oscillations. So far the few Myc factors characterized resemble Nod factors but there are likely to be other factors involved as well. The SYM genes necessary for bacterial symbiosis are the same genes required for AM symbiosis, which is probably their ancestral role.

Arbuscular mycorrhizal symbiosis – formation and function of the arbuscule (101 – 104)
Perception of strigolactones initiates branching in the fungal hyphae. When the hyphae contact the host root they form an appressorium and penetrate into the root cells. In the root cortical cells the hyphae branch extensively to form a tree-like arbuscule, which is surrounded by a plant-derived periarbuscular membrane across which nutrients are exchanged.

Ectomycorrhizal symbiosis (105 – 108)
Ectomycorrhizal (EM) fungi are facultative symbionts, and as many as 25,000 species may fall into this group. Approximately 8000 plant species are hosts for EM fungi, including most species of temperate forest trees. Many familiar and edible mushrooms are the fruiting bodies of EM fungi. EM fungi differ from the other symbionts described here in that they do penetrate between but not within plant cells. Nevertheless there is ample evidence that they contribute to the host’s nutrient uptake.

SUMMARY AND FUTURE STUDIES (110 – 116)
Symbiotic microorganisms are intimate allies of plants. These symbioses are mutualistic association in which both partners benefit. The plant uses a core SYM pathway for symbioses with mycorrhizal fungi and rhizobia. The conserved SYM pathway is involved in symbiosis with rhizobia, Frankia and AM fungi. AM symbiosis preceded nodulation by several hundred million years, therefore it is generally accepted that later pathways are derived from the AM symbiosis pathway.
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<td>14 - 16</td>
<td>Biological nitrogen fixation – only prokaryotes can do it</td>
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<td>Uses the enzyme nitrogenase and lots of ATP (16 per mol N2 fixed)</td>
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<td>Diazotrophs (nitrogen fixers) are scattered about the prokaryote kingdom and are thought to have obtained the ability to fix nitrogen through horizontal gene transfer</td>
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<td>Symbiotic nitrogen fixation</td>
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<td>The rhizobia – legume symbiosis</td>
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<td>Distribution and significance – rhizobia are diverse, defined by ability to nodulate legumes. Legumes are important sources of protein</td>
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<td>Plant-produced flavonoids induce transcription of bacterial nod genes, which produce Nod factors</td>
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<td>Nod factors are lipo-oligochitins</td>
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<td>The modifications to the Nod factor backbone determine host specificity</td>
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<td>Nod factors are perceived by nod-factor receptors and induce root hair curling</td>
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<td>Some rhizobia do not produce Nod-factors but are able to initiate nodule formation</td>
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<td>34 - 42</td>
<td>The common SYM pathway</td>
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<td>A set of genes called SYM genes are necessary for symbiosis – both with rhizobia and with mycorrhizal fungi</td>
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<td>The SYM pathway transduces information from the plasma membrane to the nucleus</td>
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<td>A calcium oscillation is at the heart of the SYM pathway</td>
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<td>The functions of some SYM genes are still being investigated</td>
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<td>43</td>
<td>Bacterial entry and nodulation process</td>
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<td>44 - 50</td>
<td>Bacteria enter through root hairs in ~ 75% of legumes</td>
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<td>Bacteria enter into the host cells through endocytosis –like process</td>
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<td>Some bacteria terminally differentiate and endoreduplicate, in a process controlled by the host</td>
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<td>51 - 57</td>
<td>Nodule morphology and development</td>
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<td>Nodule formation is regulated by hormones – cytokinin and auxin</td>
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<td>Nod-factor independent rhizobia induce nodule formation by cytokinins or related compounds</td>
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<td>Nodules are determinate or indeterminate</td>
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<td>Indeterminate nodules are zonated, determinate ones not</td>
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<td>In some plants like <em>Sesbania</em>, nodules can form on the stem</td>
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<td>58</td>
<td>Symbiotic nitrogen fixation requires teamwork</td>
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<td>The bacteria provide nitrogenase.</td>
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<td>The host plant provides leghemoglobin, homocitrate, carbon sources, organic nitrogen…..</td>
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<td>59 - 63</td>
<td>Nitrogenase is very sensitive to deactivation by molecular oxygen and must be maintained in a low O2 environment. Plants produce leghemoglobin solely for the purpose of buffering oxygen to allow nitrogen fixation to proceed</td>
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<td>64 - 67</td>
<td>Most rhizobia do not produce homocitrate, a necessary cofactor of nitrogenase, and must be supplied it by the plant. An unusual rhizobium that can fix nitrogen</td>
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independently also produces homocitrate!

68 - 69 Rhizobia switch off glutamine synthase, meaning that all of the ammonium they produce is exported to the plant. It also means that they depend upon the host for amino acids.

70 - 76 Plants control nodule formation systemically (autoregulation of nodulation) and locally. A CLV-like receptor is involved and CLE-like peptides. Nodules that don’t fix nitrogen are sanctioned and do not grow.

77 **Legumes and rhizobia - summary**

78 **Root nodule symbiosis – beyond legumes**

79 - 83 *Parasponia* is the only genus outside of legumes nodulated by rhizobia. Frankia are bacteria that nodulate diverse actinorhizal plants. Actinorhizal nodulation is thought to have an independent origin from that of legumes, is Nod-factor independent, and the nodules are modified lateral roots derived from pericycle cells (in contrast to legume nodules which are derived from cortical cells). There is evidence that the SYM pathway is necessary for this symbiosis.

84 **Root nodule symbiosis - Summary**

85 **Mycorrhizal fungi: fungi that trade soil nutrients for carbon**

86 - 88 Phosphate is an important nutrient that becomes depleted around plant roots, but in symbiosis with mycorrhizal fungi the plant can assimilate phosphate from a much larger area.

89 - 90 Arbuscular mycorrhizal fungi are a single family of endomycorrhizal fungi that associate with most plants; ectomycorrhizal fungi are very large and diverse group that are facultative endosymbionts and associate mainly with gymnosperms and angiosperms.

91 Other mycorrhizal fungi are ericoid mycorrhizal fungi and orchid fungi.

92 **Arbuscular mycorrhizal fungi – form arbuscules within plant cells**

93 - 96 The plant – AM fungi symbiosis is thought to have persisted for more than 400 million years through fossil evidence, and this is supported by genetic data (conservation of SYM genes across all plants).

97 **Signaling between AM fungi and plant**

98 - 99 Plants produce strigolactones that are recognized by AM fungi and elicit hyphal branching. AM fungi produce Myc factors (at least some of which are lipo-oligochitins that resemble Nod factors) that elicit plant responses.

100 AM signaling pathway involves GRAS transcription factors, some of which are homologous to those activated during root infection by pathogenic fungi.

101 - 104 The AM fungi branch and move into the root, eventually into the root cortex where they form arbuscules, specialized structures for nutrient exchange. The plant actively facilitates fungal entry. There is a periarbuscular membrane surrounding the arbuscule across which nutrients are transported. Both plant and fungi reward good partners.

105 **Ectomycorrhizal fungi associate with trees**

106 - 108 Many familiar and economically important mushrooms and truffles are ectomycorrhizal fungi. There numbers have been declining due to over harvesting and pollution. They have been shown to help trees stay healthy. Some have had their genomes sequenced, revealing interesting adaptations thought to enhance their ability to form symbiotic associations.

109 **Mycorrhizal symbioses - Summary**

110 - 116 **Summary and Ongoing Research**

Plants benefit from associations with their intimate allies, and so do their partners – these are truly mutualistic associations. The conserved SYM pathway used for diverse symbioses. Many molecular, evolutionary and ecological questions remain.