



BEYOND LEGUMES: ACTINORHIZAL SYMBIOSIS: THE NATURAL ART OF NITROGEN FIXATION

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Abstract

Actinorhizal symbiosis is a nitrogen-fixing interaction between *Frankia* an actinobacteria and the roots of woody dicotyledonous plants of 25 genera and other eight families. This association results in the formation of coralloid root nodules which convert atmospheric nitrogen into bioavailable forms, allowing plant growth in nitrogen-poor or degraded soils. Actinorhizal plants are effective pioneer species for land reclamation, reforestation, and phytoremediation, particularly under abiotic stresses such as drought, salinity, and heavy metals. Infection by *Frankia* occurs through root hair invasion or intercellular penetration, depending on the host. Although similar to legume nodules, actinorhizal nodules have a different developmental origin and gene expression. Recent genomic studies reveal complex signaling and specificity, highlighting the potential of these symbioses in sustainable land management and ecosystem restoration.

Introduction

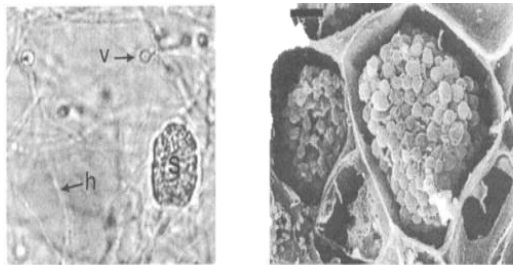
Nitrogen is essential for plant life, yet most plants cannot access it directly from the atmosphere. Remarkably, certain plants have evolved intimate symbiotic partnerships with

soil-dwelling microbes to solve this challenge. Two such systems dominate the world of biological nitrogen fixation: the well-known Rhizobium–legume symbiosis, and the lesser-known but equally fascinating actinorhizal symbiosis between actinobacteria of the genus *Frankia* and over 220 woody plant species. These associations result in the formation of root nodules—specialized plant organs where bacteria live inside root cells and convert atmospheric nitrogen into usable forms.

Actinorhizal plants, like alders and sea buckthorn, are ecological pioneers, thriving in poor, damaged, or stressed soils where few other species survive. Unlike rhizobia, *Frankia* can also fix nitrogen independently, forming complex vesicles that protect its nitrogenase. Enzyme from oxygen damage. Though both symbioses are evolutionarily distinct, they share intriguing similarities, including parallels with ancient fungal symbioses. As scientific tools advance, researchers are beginning to unlock the complex signaling, evolution, and environmental roles of these hidden partnerships—offering new hope for sustainable agriculture, reforestation, and soil restoration in an era of environmental change.

Family	Genus	Representative Species	Ecological Role
Betulaceae	<i>Alnus</i>	<i>A. glutinosa</i> , <i>A. incana</i>	Riparian zones, disturbed soils
Casuarinaceae	<i>Casuarina</i> , <i>Allocasuarina</i>	<i>C. equisetifolia</i>	Coastal, arid lands, agroforestry
Datisceae	<i>Datisca</i>	<i>D. glomerata</i>	Herbaceous, rare symbiosis
Elaeagnaceae	<i>Elaeagnus</i> , <i>Hippophae</i> , <i>Shepherdia</i>	<i>E. angustifolia</i> , <i>H. rhamnoides</i>	Soil reclamation, erosion control
Myricaceae	<i>Myrica</i> , <i>Morella</i>	<i>M. gale</i> , <i>M. cerifera</i>	Wetlands, sandy soils
Rhamnaceae	<i>Ceanothus</i> , <i>Discaria</i>	<i>C. americanus</i> , <i>D. trinervis</i>	Nitrogen-poor forest understory
Rosaceae	<i>Purshia</i> , <i>Cercocarpus</i>	<i>P. tridentata</i> , <i>C. ledifolius</i>	Dry, rocky, semi-arid ecosystems

The Biology and Symbiotic Function of *Frankia* in Actinorhizal Associations



(A) *Frankia* stain culture grown under normal oxygen tension and nitrogen limiting conditions.

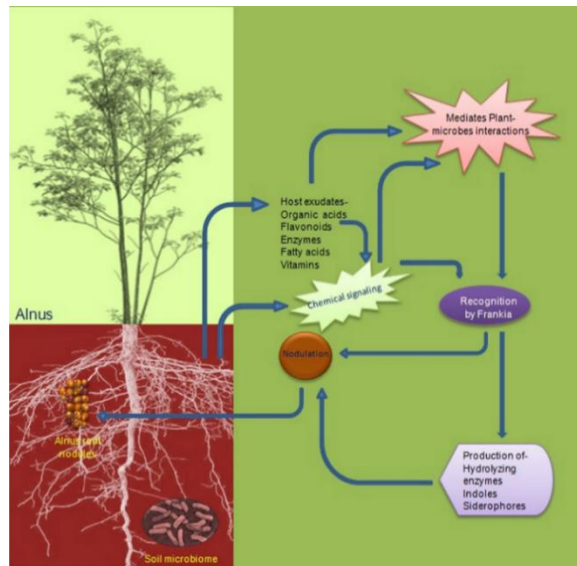
(B) Electron micrograph of an infected cell of *Alnus glutinosa* nodule

The genus *Frankia* comprises filamentous actinobacteria capable of forming nitrogen-fixing symbioses with a wide range of woody dicotyledonous plants collectively referred to as actinorhizal species. Initially described in the late 19th century and classified as an actinomycete in the early 20th century, *Frankia* was first successfully isolated in 1978 from *Comptonia peregrina* root nodules, marking a turning point in the study

of actinorhizal symbiosis. In both culture and symbiotic states, *Frankia* differentiates into three distinct cell types: vegetative hyphae, nitrogen-fixing vesicles, and multilocular sporangia.

Vesicles, the primary site of nitrogenase activity, are surrounded by multilayered envelopes rich in hopanoids, which act as a gas diffusion barrier, thereby enabling nitrogen fixation under aerobic conditions. This adaptation sets *Frankia* apart from rhizobia, most of which are obligate symbionts incapable of free-living nitrogen fixation. Frankial strains vary in sporulation capacity, with spore-forming (spore⁺) strains typically demonstrating greater infectivity than their non-sporulating (spore⁻) counterparts. Molecular tools such as 16S rRNA and *nif* gene-based markers have revealed significant genetic diversity within the genus, facilitating phylogenetic classification and host-specificity studies. Although the bacteria are slow-growing and remain genetically intractable, *Frankia* can persist in soils devoid of

actinorhizal hosts and is capable of nitrogen fixation in free-living conditions, implying broader ecological roles beyond symbiosis. As genomic and molecular resources expand, *Frankia* continues to serve as a model organism for investigating nitrogen fixation, microbial adaptation, and sustainable strategies for ecological restoration.



Ecological Role of Actinorhizal Plants

Actinorhizal plants, mainly woody perennials and a few herbaceous species like *Datisca*, play a crucial role in ecosystem stability and recovery. They form nitrogen-fixing symbioses with *Frankia* bacteria, enabling them to thrive in nutrient-poor, arid, and disturbed environments. Many species are also mycorrhizal, forming tripartite associations that enhance nutrient acquisition and stress tolerance. This combined symbiotic capability allows actinorhizal plants to colonize degraded soils, contributing significantly to nitrogen input and ecosystem restoration. Their use in agroforestry, reforestation, erosion control, and desertification prevention highlights their ecological and economic value.

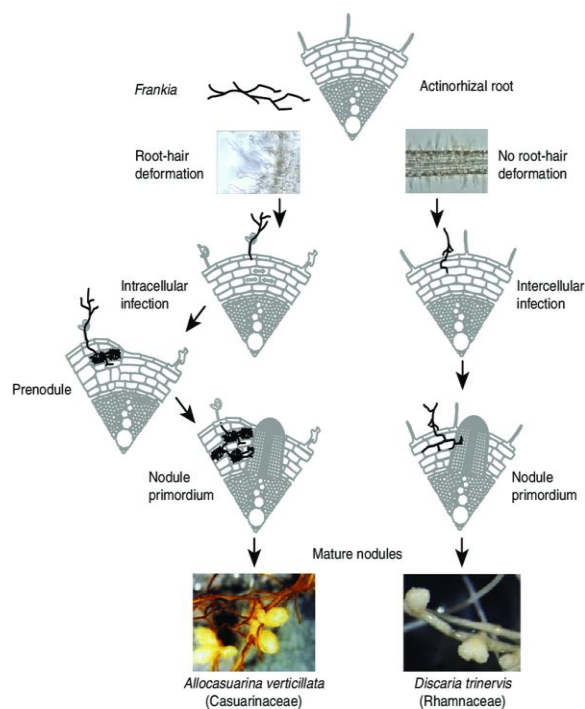
With annual nitrogen fixation rates comparable to legumes (240–350 kg N ha⁻¹

y⁻¹), actinorhizal species enhance soil fertility over time through leaf litter and organic matter decomposition. As pioneer species in ecological succession, they prepare the soil for other plant communities, making them essential tools for sustainable land management and biodiversity support in fragile and recovering ecosystems.

Structure and Gene Expression in Actinorhizal Root

Actinorhizal root nodules are indeterminately growing structures that resemble lateral roots but originate independently without forming a root cap. They consist of distinct zones: an apical meristem, infection zone, nitrogen fixation zone, and senescent zone. The fixation zone contains both infected cells with nitrogen-fixing vesicles and uninfected cells involved in nutrient exchange. Nodule anatomy and oxygen protection mechanisms vary across genera. Gene expression studies reveal both unique and legume-homologous genes, such as early nodulins and hemoglobins, indicating conserved functions. These findings support evolutionary links between actinorhizal and legume symbioses through independently regulated developmental pathways.

Infection Pathways in Legume and Actinorhizal Symbioses: Intracellular and intercellular mechanism



In actinorhizal symbioses, *Frankia* initiates infection either intracellularly or intercellularly, depending on the host plant lineage. In members of the order Fagales, the infection is predominantly intracellular, initiated by root hair deformation followed by bacterial entry through a curled root hair structure—reminiscent of rhizobial infection in legumes. In contrast, in Rosales and possibly Cucurbitales, *Frankia* employs an intercellular pathway, gaining access to the root cortex through enzymatic dissolution of the middle lamella between adjacent epidermal cells. Once inside the host, unlike rhizobia that are released into the cytoplasm, *Frankia* remains confined within plant-derived fixation threads. Within these threads, the bacteria differentiate into specialized nitrogen-fixing vesicles—structures that provide a low-oxygen environment essential for nitrogenase activity. These contrasting infection strategies reflect the evolutionary plasticity of the actinorhizal symbiosis and underscore its distinct adaptations across diverse plant lineages.

Conclusions

Actinorhizal plants hold promise for sustainable agriculture by enabling nitrogen fixation in non-leguminous species. Future efforts should focus on closely related species with economic value. Although some studies show synergistic effects between *Frankia*, mycorrhizal fungi, and other microbes, results are inconsistent. Interestingly, *Frankia* nodulation may also enhance pathogen resistance, offering benefits beyond nitrogen nutrition. However, the mechanisms underlying these complex interactions remain poorly understood. Further basic research is essential to harness the full potential of actinorhizal symbioses for improved crop productivity and ecological resilience.

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