

Frankia–actinorhizal symbiosis with special reference to host–microsymbiont relationship

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The status of current knowledge on the *Frankia*–actinorhizal plants symbioses has been reviewed with special reference to the physiology of the nodule, the plant and *Frankia* genes involved in the symbiosis, nodulation and the effects of plant, *Frankia* and the combination on the regulation of symbiosis.

SYMBIOTIC associations that develop between microorganisms and higher plants have received recognition due to their effects on plant morphogenesis, nutrition, protection against infectious diseases and study of basic cell biology. These associations cater to the nutritional needs of the biosphere and are responsible for generating almost 50% of the fixed nitrogen annually. *Rhizobium*–legume symbiosis has almost become synonymous with plant–microbe symbiosis. This is not surprising because legumes occur widely and *Rhizobium* is a fast-growing microbe, and easy to obtain in pure cultures. But there are other symbiotic systems which are equally relevant and interesting. These are *Frankia*–actinorhizal trees, *Bradyrhizobium*–*Parasponia*, *Nostoc*–*Azolla* and others. These systems are distinct and each displays characteristic similarity and differences from the *Rhizobium*–legume system. Particularly intriguing is the case of *Frankia*–actinorhizal tree symbiosis, which is the subject of this review.

The Actinomycete genus *Frankia* belongs to the recently emended family, Frankiaceae¹. Its members are Gram-positive bacteria that nodulate about eight plant families representing about 25 genera of woody, dicotyledonous, perennial angiosperms, collectively called actinorhizal plants². The term actinorhiza is given to root nodules that are formed by *Frankia*.

Actinorhizal plants are popularly used as pioneer plants in the regeneration of waste lands³. Prominent among these are *Alnus*, *Shepherdia*, *Elaeagnus* and *Hippophae*, which play a vital role in soil reconstruction. Some actinorhizal plants are used as windbreaks⁴, pulpwood⁵, timber⁶ and fuel wood⁷, while others have use in the human diet³ and as forage for livestock (*Ceanothus* and *Purshia*). *Myrica* spp. are used in traditional Indian medical system for prevention and cure of flu, common cold and others. Actinorhizal trees are also valued for

landscaping, providing shade, and contributing to the beautification of parks and cities⁷. Apart from these practical aspects, the *Frankia*–actinorhizal plant system provides enough food for thought to those interested in cell biology. The parallels between *Rhizobium*–legume and *Frankia*–actinorhizal tree systems are striking. *Rhizobium* is a Gram-negative, free-living bacterium and infects legumes only, while *Frankia* is Gram-positive and filamentous, and can nodulate a diverse range of host genera. Both *Rhizobium* and *Frankia* produce root nodules in which dinitrogen is converted to ammonia. The quantum of fixed nitrogen produced by the two systems is comparable. The *nif* genes in both bacteria share sequence homology⁸. These similarities raise many questions about the nature of symbiotic interactions in general, and *Frankia*–actinorhizal tree symbiosis in particular. For instance, why is *Frankia*, a slow-growing bacterium, able to nodulate such a diverse range of host genera, while *Rhizobium* infects only legumes? Which features are common to the host species that associate with *Frankia*? What is unique about *Frankia*–actinorhizal tree symbiosis? What are the common features with other systems? Which genes are conserved between the two systems and which genes are different? The questions are many and the answers are not forthcoming, since it is only in 1978, that the pure cultures of *Frankia* became available⁹. This means that specific tools for analysing the molecular biology cannot be easily developed. But some problems have been solved by cloning *Frankia* DNA into *E. coli*. As a consequence, some genes can be identified from a *Frankia* gene library by comparing with other genes. However, a major advance in *Frankia*–actinorhizal tree molecular biology will require the use of cloning vectors as well as the development of a transformation system. Meanwhile, another approach to the study can be to look into the salient features of host–microbe specificity and try to understand the peculiar characteristics of this partnership.

In general, a nodule is a modified lateral root. *Rhizobium*, *Bradyrhizobium* and *Frankia* infect and form nodules by different ways. *Frankia* infects the roots primarily by root-hair infection. Nodules formed have an internal anatomy similar to that of lateral roots with a cortical cylinder of vascular tissue, a cortical region in which the infected cells are found¹⁰ and a typical outer

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periderm layer. In actinorhizal nodules, a pre-nodule consisting of infected and uninfected cells is, however, formed. The nodule primordium formation does not involve pre-nodule cells. Thus the pre-nodule apparently represents a primitive symbiotic organ¹¹.

Most nodule lobes have a determinate meristem at the distal tip. *Frankia* exists in the vegetative mycelial state in the nodules. Nitrogenase enzyme is sensitive to exposure to O₂ and is protected by the formation of vesicles. The multilaminar vesicle envelope has a lipid composition differing from that of non-induced cultures and has a high amount of C₂₂–C₂₆ polyhydroxy fatty acids or alcohol. The extent of vesicle formation and shape are controlled by the host plant. However, vesicles are not found in *Casuarina* and *Allocauarina* root nodules. The lack of vesicles in *Casuarina* is not a characteristic of the micro-symbiont, since it forms vesicles in cultures. It reflects the low O₂ levels within the nodule tissue controlled by suitable modification of host cells. Cytological analysis of *Casuarina* nodules indicates that infected host cell walls change in composition upon penetration of cells by *Frankia*; they become more hydrophobic and probably less permeable to O₂. *Coriaria* nodules display a distinct mechanism for providing O₂ protection. In this genus the infected cells are surrounded by a thick periderm whose thickness varies with pO₂.

Nitrogen fixation is a process which has a high energy requirement. Actinorhizae having symbiotic associations, the microbe obtains the requisite energy by way of carbon compounds from the host plant. The exact nature of compounds furnished by the host is unclear. At least some of the *Frankia* strains may not be able to use simple sugars in pure culture¹². It is thought that these organisms lack glycolytic enzymes and obtain their carbon preferentially from lipids. Addition of long-chain fatty acids to the growth medium enhances growth several fold¹³. Maudinas and co-workers¹⁴ reported that *Alnus* nodules were in fact rich in lipids and saturated and unsaturated fatty acids, and their oxidation of these may provide the necessary energy to the endosymbiont. During the reduction of atmospheric nitrogen in the nitrogen-fixation process, protons are reduced to release hydrogen. This causes a decline in the yield, but the loss is recuperated by the use of an uptake hydrogenase. This enzyme recycles all the hydrogen that is formed. The enzyme can even oxidize atmospheric hydrogen in the soil, thus increasing the energy resources of the bacterium¹⁵. An interesting finding is the detection of Rubisco activase mRNA in the root nodules of *Datisca glomerata*¹⁶. However, no specific role of this protein could be established.

Plant genes involved in symbiosis

Plant proteins specially formed in response to plant-microbe interactions are termed as nodulins. In many

legumes about 20–30 nodulins are detected in effective nitrogen-fixing nodules. For example, in effective root nodules of alfalfa 17 nodule-specific translation products, including uricase have been identified¹⁷. Nodule-specific glutamine synthase¹⁸ and leghaemoglobin¹⁹ have also been reported. Leghaemoglobin is one of the most characteristic proteins found in legume root nodule. Approximately 20–25% of the total protein found in legume nodule comprises leghaemoglobin. A gene *cg12* from *Casuarina glauca* has been cloned and characterized recently²⁰. It was found to have a strong homology to subtilisin-like protease gene families of several plants, including the actinorhizal nodulin gene *ag12* of *Alnus glutinosa*. This gene expresses early during nodule development, and therefore, represents an early actinorhizal nodulin gene.

Nodules of a number of actinorhizal plants contain haemoglobin, but others do not. Haemoglobin is found at higher levels in *Casuarina* and *Myrica* and at lower levels in *Alnus* and *Elaeagnus*²¹. Leghaemoglobin of *Casuarina* is thought to share sequence similarity with that of *Parasponia*, but differs from that of soybean. *Datisca* spp. lack leghaemoglobin but display nitrogen-fixation rates similar to nodules of *Casuarina*²¹.

Absence or low levels of haemoglobin may be related to the other ways of restricting access of O₂. For example, *Casuarina* nodules contain relatively high levels of haemoglobin. The host cell walls surrounding the microbe are suberized²² and this may restrict O₂ diffusion to the *Frankia* cells. The large variation in the presence or absence of haemoglobin in actinorhizal plants, in the occurrence of vesicles and in suberization of cell walls, indicates that various strategies have been developed in plant-*Frankia* symbiosis to overcome the damage to nitrogenase enzyme by free O₂. The ability of angiosperms to form an association with *Frankia* is widespread amongst seemingly unrelated plant families and genera. However, based on the chloroplast gene sequence data, Soltis and co-workers²³ suggested a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. But different plant genera seem to have evolved different ways of restricting access of oxygen to the site of nitrogenase activity.

Nodule-specific glutamine synthase is understood to be present in several nodules. A majority of actinorhizal plants investigated transport asparagine or glutamine whereas others (*Alnus*) transport citrulline²⁴. However, the metabolism of nitrogenous products is still unclear.

Frankia genes involved in nodulation

Nod genes have been described in the literature. These genes are responsible for root-hair curling and cortical cell division. The common *nod* genes are *nodA*, *nodB*, *nodC* and *nodD*. These genes are located on the resident

chromosome unlike *Rhizobium*, where they are located on megaplasmids²⁵. Mutation in any of these genes results in *nod*⁻ phenotype. Recent investigations have revealed that *nod ABC* genes are responsible for the synthesis of a factor that induces the characteristic early responses in the host plant. The factor Rm1 was identified as a derivative of N-acyl-b-1,4-D-glucosamine tetrasaccharide. Other genes are *nodSUIJ*, *nodY*, *nodK*, etc. *nodD* is thought to be responsible for *nod* gene induction. Host specificity genes modify the products of *nod* genes. Recently, a gene *nolA* has been identified as genotype-specific nodulation gene that determines the infection of specific plant genotypes within a given legume species.

Because of the similarity in the early steps of nodule development between *Rhizobium* and *Frankia*, it was thought that nodulation genes between the two organisms would be conserved. However, the attempts of hybridization involving the *nod* genes of *Frankia* and *Rhizobium* have met with little success²⁶.

The process of invasion of host cells by *Frankia* may involve degradation of pectin. The genes coding for pectate lyase in *Erwinia chrysanthemi*, an enterobacterium pathogenic to many plants, share a lot of sequence similarity with *Frankia pel* genes. Similarly, cellulolytic activity is also thought to be present in *Frankia*²⁷. Presence of *hup* genes in *Frankia* has also been reported, but sequence homology with other nitrogen fixers has not yet been investigated. *Rhizobium* strains exist in two forms, either *hup*⁻ or *hup*⁺. It is thought that the *hup*⁺ strains are more efficient in fixing nitrogen²⁸. *Bradyrhizobium* genes have been found to hybridize with *Frankia hup* genes, but so far the *hup* genes have not been localized.

Regulation of symbiosis

Nitrogen fixation is overwhelmingly a fraternal endeavour. The photosynthetic activity of the plants is linked together with the nitrogen-fixation activity of bacteria to reduce dinitrogen. There is a division of labour between the two organisms, a careful orchestration of which permits optimum benefits to both. The precise nature of the regulation of this relationship is intriguing. Is it purely a one-man show? Or, is it based on equal partnership? Or, is there a third dimension in which one partner dominates while the other chooses to play a subdued but significant role?

Our knowledge of the plant genes involved in symbiosis is fragmentary as is our information about frankial genes and their expression. We can at best glance at the morphological and physiological features of some of the steps involved in this process and try to extrapolate it to the composite endosymbiotic system. It seems that the actinorhizal plants undergo feedback regulation of symbiosis. There are at least two different signals that lead to regulation of nodulation²⁹. The symbiotic relationship

between *Frankia* and actinorhizal trees seems to be under the control of mainly three components: the host genotype, the *Frankia* genotype and other elements.

Role of host

Several reports suggest that the nitrogenase activities of *Frankia* isolates are different in pure culture and *in situ* conditions. Strains displaying higher nitrogen-fixation rates in culture often show lower rates *in situ* and vice-versa. Obviously, this effect is brought about by the host. The host genotype controls nodule morphology. Furthermore, the same bacterial strain may nodulate different hosts and may inhabit nodules of different morphologies. Various strains of *Frankia* are known to produce different responses in a clone of *Casuarina equisetifolia*³⁰ and *Alnus*³¹. In our laboratory, a *Frankia* strain was found to be highly active in symbiotic condition compared to others³². This strain had low activity in culture but when different host genotypes were nodulated, the nitrogenase activity *in situ* was high. When a combination of high and low nitrogen-fixing *Frankia* strains were selected and tested on three *Casuarina* host clones, it was found that a certain host clone always produced the maximum nitrogen-fixing nodules irrespective of the strain used³³. Similarly, there was a host clone which always produced nodules with the lowest activity³³.

The physiology of the host is the machinery that is availed by the endosymbiont during the reduction of nitrogen. The demand for fixed nitrogen comes in a major way from the host. Obviously the microbe must, therefore, work to the dictates of the host. The plant may affect this control by regulating key steps in symbiosis^{34,35}. Some of these steps are: (i) recognition of specific strains at the time of infection and selection of a particular strain; (ii) development of the nodule; (iii) selective suppression of host defence genes so that the selected strain is allowed to develop while others are restricted; (iv) provision of a protective environment for functioning of nitrogenase, either by regulating the levels of leghaemoglobin, by controlling the thickness of vesicle walls or by controlling the thickness of host cell barrier; (v) controlling the metabolism of nodule especially by regulating levels of glutamine synthase, by controlling export of ammonia or by controlling levels of ATP required for nitrogen fixation, and (vi) regulation of the synthesis of carbon compounds for utilization by the microsymbiont.

Role of Frankia

During infection, *Frankia* cells are known to elicit protein factors that mediate the selection of the host for infection. Though several strains of *Frankia* may infect simultaneously, only a few are known to form nodules; of these, a few produce effective nodules. During the entire

process of symbiosis, a continuous interplay between frankial determinants and the host-related phytohormones ensues. The host and the microbe also mutually enhance their individual characters³⁶. This regulatory process is modulated by temperature, nitrate concentration in the soil, moisture and other environmental factors.

The central fact, however, is that *Frankia* is the contributor of the nitrogen-fixation apparatus. This makes it a direct player in the process of nitrogen reduction. Obviously, strains that have an efficient nitrogenase system would produce more fixed nitrogen compared to others when all other factors are constant. The role of the microsymbiont is thus vital in nitrogen fixation. Screening of a large number of isolates under diverse environmental conditions and use of different host genotypes, can help in identification of superior nitrogen-fixing *Frankia* strains. By following such a strategy, Han and New³⁷ were able to obtain a single high nitrogen-fixing strain of *Azospirillum*, among a collection of 285 strains. Similar results were recorded in case of *Rhizobium* by Batzli and co-workers³⁸. However, our attempts at identifying superior *Frankia* genotypes using molecular markers met with a limited success³⁹. *Frankia* genotype, therefore, is a major player in the success of symbiosis, but appears to be greatly influenced by other factors.

Combined effects

The functional relationship between *Frankia* and plant is far from simple. The microbe and plant may show complete compatibility as far as establishment of infection is concerned, but the resulting association may not provide

optimal benefit to either partner. The physiological activity of the two partners appears to have a role in determining the functional compatibility. This compatibility is a prerequisite for the phenotypic expression of the frankial association (frankial effectiveness) and depends not only on the genetic make up of *Frankia* and the plant involved, but also on factors external to the association. External factors have the potential to bring about an imbalance in the symbiotic relationship. The role of pH, soil, soluble phosphorus, calcium levels, available nitrogen, conductivity of water, climate, light availability, canopy cover, etc. are significant in this regard^{37,40-43}. Significant variations in acetylene reduction assay values with changes in the parameters mentioned above were reported. Other factors that may similarly influence the outcome of symbiosis are age of the tree, age of the nodule and presence of other microbial flora in the vicinity of the nodule.

Conclusions

The symbiotic efficiency of partnership between the actinorhizal host and the *Frankia* strain seems to be defined broadly by the functional capacity of the association and environmental factors. The functional capacity is itself a function of the host genotype, the *Frankia* genotype, and the environmental factors (Figure 1). Though large gaps still exist in our understanding of the intricacies of this symbiotic process, a considerable progress has been made in recent years in several aspects of *Frankia* physiology, the physiology of the host and their molecular biology and genetics. Further advances in the subject may enable a better understanding of how this system functions. It would then be possible to envisage the possibility of a better utilization of the symbiosis to improve the production of actinorhizal species in forestry.

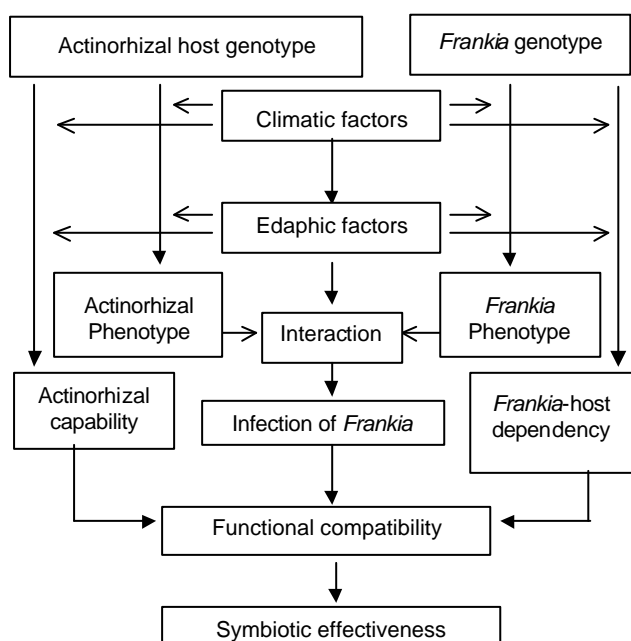


Figure 1. Complexity of host-*Frankia* interaction and symbiotic effectiveness.

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Erratum

Mega-geomorphology and sedimentation history of parts of the Ganga–Yamuna plains

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The course of the Ganga river in this region has been described as a zone of subsidence that demarcates the boundary between the tectonically uplifted block situated on the southern bank of the river with north-facing escarpment and the northern block with extensive floodplain. A reconnaissance hydrogeomorphic map of this

region using Landsat images identified three distinct divisions as upland tract, ravinous tract and floodplain¹.

Should read as:

The course of the Ganga river in this region has been described as ‘a zone of subsidence that demarcates the boundary between the tectonically uplifted block situated on the southern bank of the river with north-facing escarpment and the northern block with extensive floodplain’^{1a,b}. A reconnaissance hydrogeomorphic map of this region using Landsat images identified three distinct divisions as upland tract, ravinous tract and floodplain^{1b,c}.

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