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New and Unusual Microorganisms and Niches

Stem-Nodulating Rhizobia

B. L. DREYFUS, D. ALAZARD, AND Y. R. DOMMERGUES
Laboratoire de Microbiologie des Sols, ORSTOM B.P 1386, Dakar, Senegal

In symbiosis with *Rhizobium* sp., most leguminous plants form nitrogen-fixing nodules on their roots. Only a few legume species also bear nodules on their stems. Stem nodulation was first reported in *Aeschynomene aspera* (17), *A. paniculata* (28), and *Neptunia oleracea* (25) and later in *A. indica* (2, 30, 29), *A. elaphroxylon* (19), *A. evenia*, and *A. filosa* (4).

Recently, Dreyfus and Dommergues (10, 11) reported the discovery of profuse stem nodulation in *Sesbania rostrata*, a fast-growing annual tropical legume which can reach a height of 3 to 5 m within 3 to 4 months. The stem nodules of *S. rostrata* were found to actively fix N_2 and to be induced by a specific strain of *Rhizobium* sp. able to form nodules on both stem and roots. Stem-nodulating rhizobia have also been isolated from stem nodules of *A. aspera* (26), *A. indica* (14; D. Alazard, unpublished data), *A. afraspera*, *A.* ~~*crassicaulis*~~, *A. elaphroxylon*, *A. schimperi*, *A. sensitiva* (D. Alazard and B. L. Dreyfus, 5th Int. Symp. Nitrogen Fixation, Noordwijkerhout, The Netherlands, 1983), and *Neptunia oleracea* (B. Dreyfus, unpublished data). Stem nodulation has been reported in other *Aeschynomene* species, namely, *A. denticulata*, *A. pratensis*, *A. rudis*, and *A. scabra* (14).

All known stem-nodulated legumes belong to the three genera *Sesbania*, *Aeschynomene*, and *Neptunia*, and have in common the ability to grow in waterlogged soils. In nature, the stem nodules of these plants are often restricted to the submerged stem and just above the flood water level.

The aim of this paper is to review the available information on stem nodulation, with special emphasis on the unusual properties of the infecting rhizobia.

STEM NODULATION SITE

The most distinctive characteristic of all stem-nodulating legumes is the presence of predetermined nodulation sites on the stems. The formation of these sites is independent of the *Rhizobium* infection. Until recently, the nature of the nodulation site was not clearly defined. Some authors have proposed that it is a lenticel in *S. rostrata* (11) and *A. paniculata* (28). Others have suggested that infection occurs on emerging adventitious roots appearing on submerged stems (2, 17, 26). Recent studies show that the infection is always initiated at the base of preformed incipient primordia on the stem. These primordia are in a dormant state, most likely as a result of a phytohormonal effect. Depending on the host plant, either they remain under the cortex or they are hidden by an external structure (lenticel or epidermal dome) or slightly pierce this structure, showing a protruding dormant apex. Anatomical study shows that these dormant, protruding or hidden primordia exhibit a typical root structure, a fact confirmed by the ability of these primordia to develop into adventitious roots when stems are immersed in water (13, 14). For that reason we call these structures dormant root primordia or root primordia. We shall see later that the distinction between protruding and hidden root primordia is important because this character governs the host sensitivity to rhizobial infection.

In *S. rostrata* (Fig. 1A) nodulation sites are distributed evenly in three or four vertical lines all along the stem (11, 13). The root primordium always pierces the stem epidermis, emerging 0.1 to 0.3 cm from an epidermal dome and forming a circular fissure (Fig. 2). Since the root primordia are protruding, the aerial infection by *Rhizobium* sp. occurs readily, producing the nitrogen-fixing nodules. The nodulation sites of *S. rostrata* are formed continuously throughout the growth of the stem and remain sensitive to *Rhizobium* infection during the whole life of the plant. In the absence of *Rhizobium* infection, the dormancy of the root primordia can be broken by immersing the stem in water, which induces adventitious roots, as indicated above, or by placing an internodal stem cutting into a nutrient medium (M. Barreto, personal communication). In the latter case, the upper primordium of the cutting develops into a shoot bud, indicating the triple potential of the nodulation site.

The genus *Aeschynomene* includes species with nodulation sites ranging from the *Sesbania* type with protruding root primordia to the hidden root primordia type. Stem-nodulated *Aeschynomene* can be divided into three subgroups.

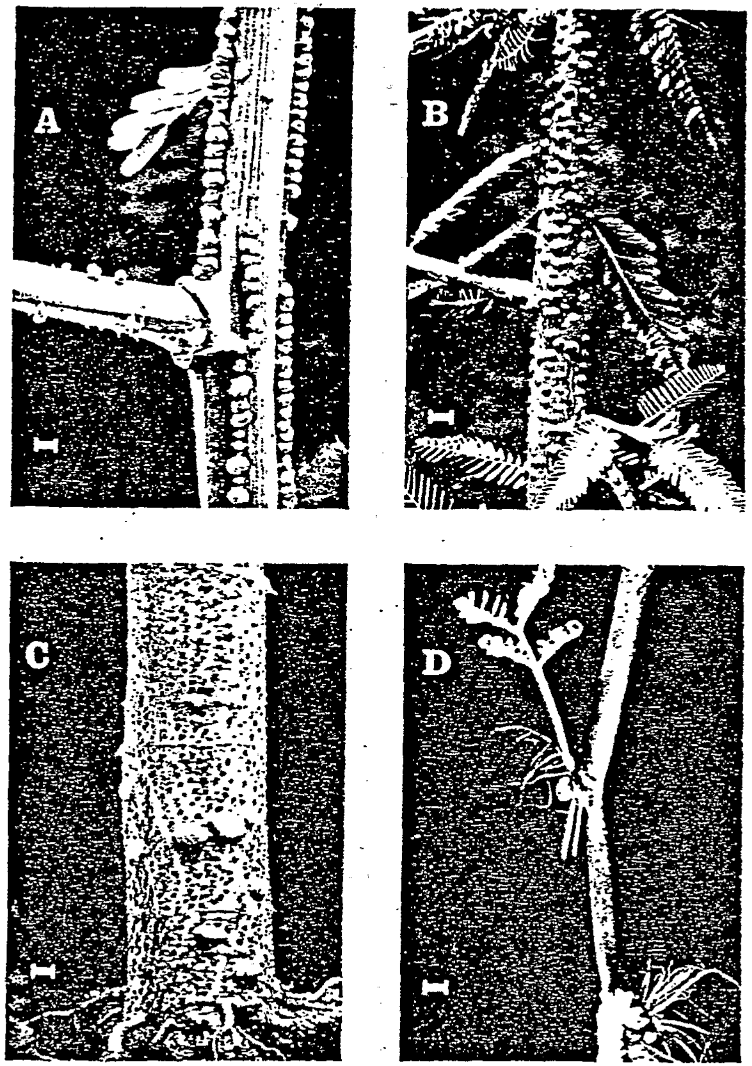

FIG. 1. Different types of stem-nodulated legumes. (A) *S. rostrata*, the most evolved type, with a profusion of nodules distributed along vertical lines. (B) *A. afraspera*, also an evolved type of stem-nodulated legume, with profuse nodulation. (C) *A. elaphroxylon*, the least evolved *Aeschynomene* sp., with a few nodules at the base of the stem. (D) *N. oleracea*, with nodules occurring only on developed adventitious roots formed at the level of the stem node. In all cases the bar represents 0.5 cm.

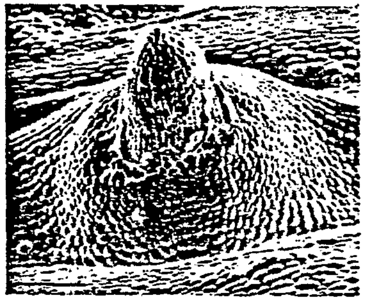

FIG. 2. Uninoculated infection site of *S. rostrata*. The root primordium always protrudes through the epidermis, forming a fissure that can be subsequently invaded by rhizobia. The bar represents 25 µm. From Duhoux and Dreyfus (13).

Subgroup 1 comprises species with the most evolved root primordia, as susceptible to infection as those of *S. rostrata*. *A. afraspera* is of this type (Fig. 1B) (D. Alazard and E. Duhoux, unpublished data).

Subgroup 2, which can be considered as intermediate between subgroups 1 and 3, comprises species with root primordia less developed than those of subgroup 1. The root primordia scarcely penetrate the epidermis and are often located in the center of lenticels. Two typical species of this subgroup are *A. scabra* (14) and *A. indica* (14; Alazard and Duhoux, unpublished data). Their root primordia are still accessible to *Rhizobium* infection, and these species nodulate, though less readily than *S. rostrata* or *A. afraspera*. *A. paniculata* and *A. sensitiva* (14, 28) probably also belong to the same subgroup. Like *A. scabra* and *A. indica*, *A. paniculata* was reported to have lenticels (28).

Subgroup 3 comprises species with the least evolved root primordia. Two typical species are *A. crassicaulis* and *A. elaphroxylon* (Fig. 1C), the latter species being an aquatic tree (26; Alazard and Duhoux, unpublished data). In these species, stem nodules are restricted to the lower and submerged section of the stem, where the conditions break the dormancy of the root primordia and induce their development into adventitious roots susceptible to rhizobial infection.

In contrast to *S. rostrata* and all the *Aeschynomene* species mentioned above, the nodulation sites of *N. oleracea* are located only in the vicinity of the stem nodes. When the plant grows on drained soils, the root primordia remain embedded in the stem cortex and no nodules are formed. When *N. oleracea* grows in waterlogged soils, the stems float at the surface of the water, and the root primordia pierce the stem epidermis and develop into typical adventitious roots, which are then susceptible to infection by *Rhizobium* sp. Nodules are usually found at the base of these roots (Fig. 1D).

Thus, differences in the structure of the nodulation site appear to explain the variations observed in the stem-nodulating ability of different species. Stem-nodulating legumes form a continuum between those with the most developed root primordia (e.g., those of *S. rostrata* and *A. afraspera*) and those with the least evolved ones (those of *A. elaphroxylon* and *N. oleracea*).

STEM INFECTION BY *RHIZOBIUM* SP.

Root nodulation has been very well documented (e.g., in Dart's excellent review [8]), but it is only recently that studies on stem nodulation have been initiated. Since these investigations concerned mainly *S. rostrata* (27) and little is known about the infection process in *Aeschynomene* and *Neptunia* species, the present discussion will be devoted mainly to the development of stem nodules in *S. rostrata*.

Before dealing with this specific topic, it is necessary to review briefly our current knowledge on the initiation of root nodules. Two different types of root infection by *Rhizobium* sp. have been reported for root nodules. In most legumes, especially temperate legumes, the infection process starts in root hairs, with the formation of an infection thread. The infection thread containing the *Rhizobium* cells passes through the root hair into the root cortex. After the infection threads have entered the meristematic cells of the cortex, the rhizobia are released into the cytoplasm of the host cells (8, 22). The second type of root infection observed in a few tropical legumes, such as *Arachis hypogaea* (6), starts by direct intercellular infection. The rhizobia penetrate at the lateral root junctions between the basal cells of root hairs and proliferate between these cells, forming intercellular zones of infection. This intercellular phase is followed by an intracellular invasion which starts with the invagination of the plant cell wall. Infection threads are not formed. The intracellular infection then develops by successive divisions of host cells, with each daughter cell receiving the rhizobia (1, 6). Similar to the *Arachis* type of infection is that of *Stylosanthes* (7), where direct cell invasion occurs but root hairs are not involved and rhizobia do not form intercellular zones of infection as in *A. hypogaea* (7). The *Arachis* and *Stylosanthes* (both in the same tribe as *Aeschynomene*) types of infection thus differ from the typical temperate legume infection by two characters: absence of root hair penetration and absence of infection threads.

In *S. rostrata* (27) the rhizobia reach the base of root primordium through the fissure encircling it. This fissure provides an optimal microenvironment for the appropriate specific *Rhizobium* sp. which colonizes it. Nodule genesis then consists of three distinct stages.

(i) Intercellular infection. The rhizobia penetrate the intercellular space of the root primordium basal cells where they multiply in large numbers, forming pockets of infection (Fig. 3). At the same time, differentiation of some cortical cells of the root primordium occurs through an activating mechanism which is still not known. The resulting meristematic cells are then infected by the rhizobia. No root hairs have been observed either in the fissure or on the root primordium.

(ii) Development of infection threads. The large intercellular pockets (up to 50 *Rhizobium* cells wide) progressively get narrower and narrower in a funnel-like manner, ending in an infection thread (one or two cells wide) which divides and penetrates the meristematic cells.

(iii) Intracellular infection. Rhizobia are released from the infection threads into the cell cytoplasm and are promptly individually surrounded by the membrane envelope (Fig. 4). At this stage (4 to 5 days after inoculation), the rhizobia (then at the bacteroid stage) begin to fix N_2 symbiotically, and the nodule exhibits the red color characteristic of leghemoglobin. Bacteroids which are originally alone in the membrane envelope later divide so that there can be up to 20 *Rhizobium* cells per membrane envelope (11). The mode of infection of *S. rostrata* stems is thus unique among the known legumes, as it involves both an intercellular invasion by *Rhizobium* sp. similar to that of *Arachis hypogaea* (6) and the development of infection threads as in temperate legumes.

In several *Aeschynomene* species the mode of infection seems to be related more to that of *Stylosanthes* sp. than to that of *Arachis* sp. since neither root hairs nor infection threads are found, and the rhizobia are spread by cell division after direct intercellular infection (2, 21).

In *N. oleracea*, according to Schaede (25), nodules are formed from infection threads initiated at the base of adventitious roots. No root hairs are found. The mode of infection in *N. oleracea* could thus be similar to that of *S. rostrata*.

The question has been raised as to whether rhizobia could reach the nodulation sites from the roots via the stem vascular bundles (17). Such an infection has never been found.

In nature, nodulation sites of *S. rostrata* are irregularly infected so that nodules appear to be distributed at random along the stem and branches. Dust seems to play a significant role in the nodulation of stems, as plants that grow along dirt roads are generally more nodulated than other plants. Other vectors that may be significant include insects and rain. Since only some of the nodulation sites are spontaneously infected, the number, and consequently the total weight, of stem nodules varies from one plant to another. Inoculating the stems greatly increases the number and weight of stem nodules of *S. rostrata*, so that nearly all the nodulating sites are nodulated (Fig. 1A).

Inoculation of *Aeschynomene* sp. of subgroup 1 always results in significant improvement of nodulation. Repeated and heavy inoculation is required for maximum nodulation of *Aeschynomene* sp. of subgroups 2 and 3.

MATURE STEM NODULE AND ITS LEGHEMOGLOBIN CONTENT

Two days after stem inoculation, there are structural changes in the root primordia of *S. rostrata* (27). Within 1 week, one can recognize the typical morphology of nodules and detect acetylene reduction activity (11). Stem nodules of *S. rostrata* are generally spherical, sometimes irregular. Except for their green chloroplast-containing cortex, they resemble typical round or oval root nodules found on *Glycine max* or *Vigna unguiculata* (11). Their diameter ranges from 0.3 to 0.8 cm, and they are easily detachable from the stem. In contrast to stem nodules, root nodules appear elongated, with an apical meristem. Plants of *S. rostrata* 3 to 4 m high can bear stem nodules of up to 40 g of fresh weight (8 g of dry weight), whereas the fresh weight of root nodules reaches only 2 to 4 g per plant.

In *Aeschynomene* spp., nodules were reported to appear within 8 days (*A. scabra*) or 12 days (*A. denticulata*, *A. indica*, *A. pratensis*, *A. rudis*, and *A. evenia*) after stem inoculation (14). Stem nodules of *Aeschynomene* are usually ellipsoidal (ca. 4 by 5 mm) or spherical (3 to 7 mm in diameter). They form more or less prominent swellings under the stem epidermis and are not easily detached from the stems. The dry weight of stem nodules of *A. scabra* can reach 0.5 g per plant (14).

Stem nodules of *N. oleracea* are elongated as a result of the presence of an apical meristem and measure up to 12 mm long. Unlike other stem nodules, those of *N. oleracea* do not harbor chloroplasts in their cortex.

In *S. rostrata* and *Aeschynomene* spp., the green cortex with chloroplasts surrounds the red-pigmented central zone. As expected, the red pigment of stem nodules was shown to be leghemoglobin (R. P. Legocki, A. R. J. Eaglesham, and A. A. Szalay, *in* A. Puhler, ed., Proceedings of the First International Symposium on Molecular Genetics of the Bacteria-Plant Interaction, in press; D. Bogusz, personal communication). In *A. scabra* (Legocki et al., in press), there are two distinct molecular species of leghemoglobin, designated Lbα and Lbβ. Stem and root nodules of *A. scabra* contained the same amount of Lbα, but Lbβ was substan-

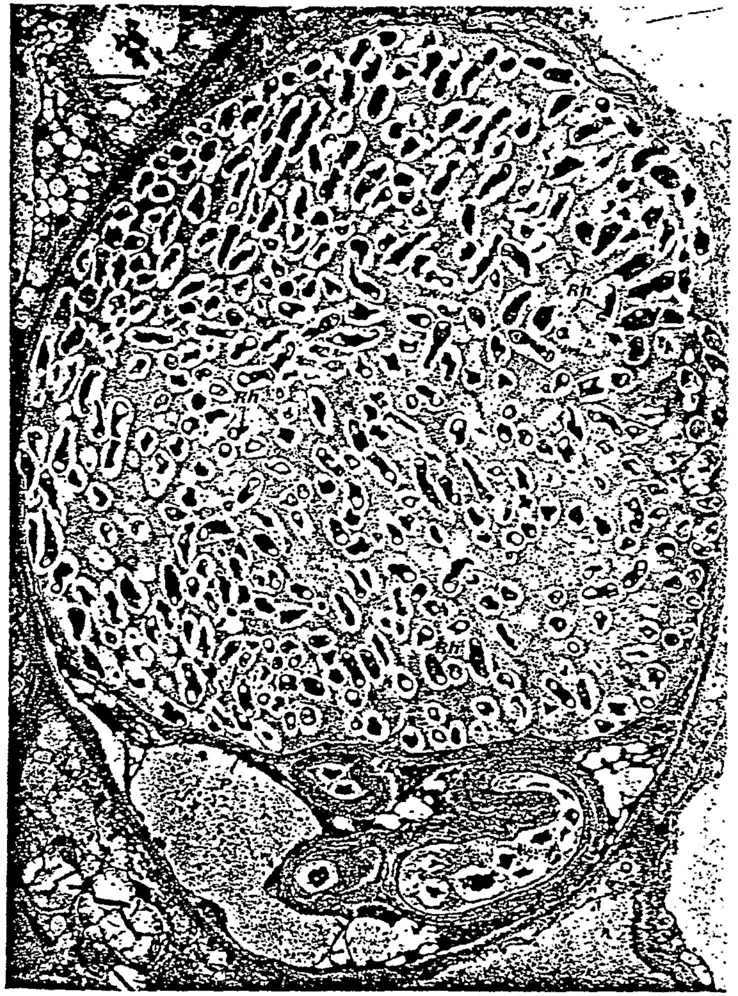

FIG. 3. Intercellular infection in *S. rostrata*. Multiplication of rhizobia (Rh) in intercellular spaces between the basal cells of the root primordium. The bar represents 5 µm. (Micrograph kindly supplied by H. C. Tsien.)

FIG. 4. Intracellular infection in *S. rostrata*. Intercellular infection threads (It) penetrate into the host cells where the rhizobia are released and immediately enclosed in the membrane envelope (Me). The bar represents 5 μm. From Tsien et al. (27).

tially more abundant in stem nodules. Recently, Bogusz (personal communication) found that the leghemoglobin from stem nodules of *S. rostrata* was composed of four different molecular species, whereas that of root nodules was composed of only three. Three root molecular species were common to stem and root nodules, and the supplementary fourth molecular species (called IIIS) was specific to the stem nodules. The differences in leghemoglobin composition between stem and root nodules of both *S. rostrata* and *A. scabra* might be in relation to the increased oxygen level resulting from the photosynthetic activity in the cortical tissues of stem nodules.

NITROGEN FIXATION

Nitrogen fixation by stem nodules of *A. scabra* and *S. rostrata* was measured by the acetylene reduction method (18). The specific activity

of stem nodules was very similar in the two species, 259 μmol of C_2H_4 g^{-1} (dry weight) h^{-1} for *S. rostrata* (11) and 270 μmol of C_2H_4 g^{-1} (dry weight) h^{-1} for *A. scabra* (Legocki et al., in press). These activities are comparable to those of root nodules of other legumes such as soybean (20) or cowpea (3). When expressed on the whole plant basis, acetylene reduction activity was 600 and 165 μmol of C_2H_4 per plant per h in *S. rostrata* and *A. scabra*, respectively. The higher activity found in *S. rostrata* is easily explained by its greater size at the time of evaluation of acetylene reduction activity. Nitrogen fixation by field-grown *S. rostrata* was shown to be about 200 kg ha^{-1} for a period of 7 weeks (24), indicating that this is one of the most powerful nitrogen-fixing legumes. Because of this potential, *S. rostrata* has been successfully used as green manure in paddy fields in Senegal, increasing the rice grain yield two- to threefold.

Combined nitrogen is well known to affect nodulation and nitrogen fixation of root-nodulated legumes, through processes that are not yet fully understood except perhaps for infection, which is known to be locally inhibited by NO_3^- (23). When grown in the presence of combined nitrogen (6 mM) in hydroponic solution or 200 kg of N ha^{-1} in soil, stem nodulation and related nitrogen fixation were not affected, whereas root nodulation was completely inhibited (9). Higher concentrations of combined nitrogen (10 to 15 mM) did not reduce the number of nodules but did affect their weight and nitrogenase activity (G. Rinaudo, personal communication). Similarly, Eaglesham and Szalay (14) found that, in *A. scabra*, N concentrations of 17 mM did not alter the number of nodules but strongly inhibited their growth and nitrogenase activity. Other *Aeschynomene* species appeared to be less tolerant to combined nitrogen than *A. scabra*, since no nodules were found when the same concentration (17 mM) was used (14).

The threshold of tolerance to combined nitrogen thus appears to be much higher in *S. rostrata* and some other stem-nodulated legumes than in legumes that have only root nodules. This characteristic confers to these stem-nodulated legumes a specific agronomic advantage.

STEM-NODULATING RHIZOBIA

Host specificity. Since we never found any cross-inoculation between rhizobia nodulating stems of *S. rostrata*, *Aeschynomene* spp., and *N. oleracea*, we propose to classify the stem-nodulating strains according to their host relatedness.

***S. rostrata* group.** Rhizobia from *S. rostrata* stem nodules (type strain ORS571) are fast growers (generation time, 3 h) but exhibit many physiological characteristics of the cowpea group (Garcia et al., in preparation).

***Aeschynomene* group.** From a study of 15 tropical strains of rhizobia isolated from stem nodules of different *Aeschynomene* species, Alazard and Dreyfus (5th Int. Symp. Nitrogen Fixation, 1983) suggested that the *Aeschynomene* rhizobia be classified into three subgroups.

Subgroup 1 contains rhizobia isolated from *A. afraspera*, which seem to have a narrow host range since they were found to effectively nodulate only *A. afraspera*.

Subgroup 2 contains rhizobia from *A. indica*, *A. schimperi*, and *A. sensitiva*, which form a relatively homogeneous cross-inoculation subgroup. Strain BTAi1, isolated from stem nodules of *A. indica* (Legocki et al., in press), probably belongs to this subgroup. Strain BTAi1, a fast-growing *Rhizobium* strain, was shown to be an intermediate type of *Rhizobium* sp. with characteristics of both fast and slow growers (M. D. Stowers and A. R. J. Eaglesham, J. Gen. Microbiol., in press).

Subgroup 3 contains rhizobia from *A. elaphroxylon* and *A. crassicaulis*, probably related to the cowpea group, since they are typically slow growers (generation time, 10 h) and effectively nodulate the roots of *Macroptilium atropurpureum*, the test host for the cowpea rhizobia.

This classification of *Aeschynomene* rhizobia fits reasonably well with the classification of their hosts of origin based on the structure of the infection site.

***Neptunia* group.** The *Neptunia* rhizobia are fast growers and probably are closely related to *R. meliloti* since they nodulate alfalfa, though ineffectively, and they contain a megaplasmid of the same size as that of *R. meliloti* (C. Rosenberg, personal communication).

Root-stem specificity. In *S. rostrata*, two types of strains have been isolated: stem-nodulating strains (called stem strains), capable of nodulating both stems and roots, and root-nodulating strains (called root strains), which nodulate only roots (Dreyfus, unpublished data). Both types are fast growers, but according to a taxonomic study (Garcia et al., in preparation), stem strains are closer to the cowpea rhizobia than to the root strains. These results suggest the involvement of specific stem-nodulating genes in the stem strains, but to date there is no information on what these genes might encode for. Since stem nodulation is dependent on the presence of roots (as dormant root primordia), this root-stem specificity is surprising. Furthermore, this specificity seems to be restricted to *S. rostrata*, since it has not been observed in other stem-nodulated legumes.

Nitrogen-fixing growth of the stem-nodulating rhizobia. For many years, it was thought that rhizobia fixed atmospheric N_2 only as bacteroids in the nodules. In 1975, several laboratories (see Gibson's review [16]) simultaneously discovered that the cowpea *Rhizobium* sp. strain 32H1 could produce nitrogenase in culture when grown under very low oxygen tension (1 µM dissolved O_2). Later, this faculty was found in a few other strains, all of the slow-growing type. In spite of their nitrogenase activity, none of these strains was able to grow solely on N_2; addition of combined nitrogen was required. Recently, we reported that strain ORS571 from stem nodules of *S. rostrata* not only exhibited high rates of nitrogenase activity in culture but also presented the unique property among rhizobia of growing in a nitrogen-free liquid or solid medium, at reduced pO_2 (12). Since then, C. Gebhardt, G. L. Turner, B. Dreyfus, and F. J. Bergersen (J. Gen. Microbiol., in press) were able to grow strain ORS571 as an N_2-fixing continuous culture. According to these authors, under optimal conditions of O_2 supply (9 µM dissolved O_2), nitrogenase activity reached 2,000 nmol of C_2H_4 mg^{-1} (dry weight) h^{-1}, and strain ORS571 showed a greater tolerance for dissolved O_2 (9 µM) than strain CB756 (1 µM) (5). This high tolerance to O_2 could result from the adaptation of the *Rhizobium* sp. to the relatively high pO_2 in the stem nodules in relation to the photosynthetic activity of chloroplasts in the cortex. N_2 fully supported the growth of ORS571, but nicotinic acid was required as a growth factor at a rate about 10 times higher than that required in the presence of combined nitrogen (C. Elmerich, B. Dreyfus, and J. P. Aubert, FEMS Microbiol. Lett., in press).

Recently, we have shown (Alazard and Dreyfus, 5th Int. Symp. Nitrogen Fixation, 1983) that tropical *Rhizobium* strains isolated from stem nodules of *Aeschynomene* subgroups 1 and 2 also exhibited significant nitrogenase activity in culture in the absence of combined nitrogen at an O_2 tension lower than for *Sesbania* sp. strain ORS571 (2 µM dissolved O_2). Thus, the ability to grow on N_2 in culture could be a widespread feature among stem-nodulating rhizobia.

Genetic analysis of nitrogen fixation in the stem-nodulating rhizobia. Following studies which have elucidated physiological traits of stem-nodulating rhizobia, molecular genetics investigations have recently been initiated in several laboratories.

Taking advantage of the property of *Sesbania* sp. strain ORS571 to grow on N_2 as the sole nitrogen source, Dreyfus et al. (12) obtained Nif^- mutants of strain ORS571 by using standard genetic methods. These mutants, unlike the parent strain, were unable to grow on agar plates under conditions of nitrogen fixation and did not fix nitrogen in the nodules of *S. rostrata*. Concomitantly, by hybridizing the total DNA of strain ORS571 with the *nif* KDH probe of *Klebsiella pneumoniae*, Elmerich et al. (15) could detect the presence of nitrogen fixation (*nif*) genes on a 13-kilobase *Bam*HI fragment. This fragment was cloned in vector pRK 290 to yield plasmid pRS1. The pRS1 plasmid was introduced into the *nif* mutants by conjugation, and genetic complementation was observed in one of the Nif^- mutants in both free-living and symbiotic states.

By hybridization with *nif* KDH of *K. pneumoniae*, *nif* genes were located by Legocki et al. (in press) on 28- and 6-kilobase *Eco*RI fragments of *A. indica* strain BTAi1. A 6.7-kilobase KDH part of the 28-kilobase fragment was cloned and used for isolation of nitrogenase structural genes and their promoters. To the best of our knowledge, Nif^- mutants of *Aeschynomene* strains have not yet been obtained.

Up to now, attempts to detect megaplasmids have been unsuccessful either in strain ORS571 (C. Rosenberg, personal communication) or in strain BTAi1 (A. Szalay, personal communication), which might mean that *nif* and possibly *nod* genes are located on the chromosome of these stem-nodulating strains, despite their being fast growers.

CONCLUSIONS

Rhizobia specific to stem-nodulated legumes differ in two respects from other known rhizobia: (i) their nitrogenase appears to be well protected against O_2, which could be an adaptation to their location in a photosynthetic nodule, and (ii) they are able to grow in the free-living state at the expense of N_2, which may confer upon these bacteria a selective saprophytic advantage.

In stem-nodulated legumes such as *S. rostrata* and *Aeschynomene* sp. of group 1, nodulation sites are well above the soil and water level, which circumvents the problems of competition from indigenous rhizobia when the stems are inoculated.

The unique characteristic of stem-nodulated plants is the presence of predetermined nodulation sites. Each site is composed of a dormant root primordium whose base is infected by the rhizobia. The structure of the nodulation site varies between two extreme types, forming a continuum between the most evolved type (*Sesbania* and *A. afraspera*) and the least evolved (*A. elaphroxylon* and *N. oleracea*). Interestingly, some Asian soybean cultivars produce dormant primordia on the lower stem, and when submerged, these primordia form adventitious roots susceptible to rhizobial infection at the stem-root junction (A. R. J. Eaglesham and A. Ayanaba, *in* N. S. Subba Rao, ed., *Selected Topics in Biological Nitrogen Fixation*, in press). This observation suggests that root primordia are not restricted to typical stem-nodulated legumes and that, by breeding or manipulating the plant genome, one could possibly induce other legumes to produce root primordia on their stems, making them susceptible to infection by rhizobia.

Such an achievement would have important implications in agriculture because stem nodulation confers upon the host several advantages. In waterlogged soils low oxygen levels are well known to reduce or inhibit root nodulation of legumes. Stem-nodulated legumes therefore have the advantage in having functional nodules in the air or at the surface of the water where the oxygen tension is still satisfactory. Since the stem nodule is a photosynthetic organ, presumably less energy is drained from the leaves to the N_2 fixation sites, so that stem-nodulated legumes are probably more efficient than other legumes. Besides these characteristics, stem-nodulated legumes have the unusual capability of actively fixing N_2 even in the presence of high rates of combined nitrogen in the soil, which allows more nitrogen to be added to and less removed from the cropping system.

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