

## Diversity of *Frankia* Strains in Root Nodules of Plants from the Families Elaeagnaceae and Rhamnaceae

MICHAEL L. CLAWSON,<sup>1</sup> MARGARITA CARÚ,<sup>2</sup> AND DAVID R. BENSON<sup>1\*</sup>

*Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269-3044,<sup>1</sup> and Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile<sup>2</sup>*

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Partial 16S ribosomal DNAs (rDNAs) were PCR amplified and sequenced from *Frankia* strains living in root nodules of plants belonging to the families Elaeagnaceae and Rhamnaceae, including *Colletia hystrix*, *Elaeagnus angustifolia*, an unidentified *Elaeagnus* sp., *Talguenea quinquenervia*, and *Trevoa trinervis*. Nearly full-length 16S rDNAs were sequenced from strains of *Frankia* living in nodules of *Ceanothus americanus*, *C. hystrix*, *Coriaria arborea*, and *Trevoa trinervis*. Partial sequences also were obtained from *Frankia* strains isolated and cultured from the nodules of *C. hystrix*, *Discaria serratifolia*, *D. trinervis*, *Retanilla ephedra*, *T. quinquenervia*, and *T. trinervis* (Rhamnaceae). Comparison of these sequences and other published sequences of *Frankia* 16S rDNA reveals that the microsymbionts and isolated strains from the two plant families form a distinct phylogenetic clade, except for those from *C. americanus*. All sequences in the clade have a common 2-base deletion compared with other *Frankia* strains. Sequences from *C. americanus* nodules lack the deletion and cluster with *Frankia* strains infecting plants of the family Rosaceae. Published plant phylogenies (based on chloroplast *rbcL* sequences) group the members of the families Elaeagnaceae and Rhamnaceae together in the same clade. Thus, with the exception of *C. americanus*, actinorhizal plants of these families and their *Frankia* microsymbionts share a common symbiotic origin.

Nitrogen-fixing symbionts belonging to the actinomycete genus *Frankia* inhabit root nodules of woody dicots from eight families of angiosperms (3). The plants colonize soils during the early stages of plant succession (2, 3, 38). Representatives of these “actinorhizal” plants are distributed on every continent, except Antarctica (2). Although some actinorhizal plants are found in tropical and subarctic regions, most species inhabit temperate zones. They grow in a variety of environments, including range lands, bogs, sand dunes, salt marsh borders, xeric chaparral, alpine regions, alluvial regions, and arctic tundra (2, 3, 31).

Actinorhizal plants and legumes, together with many non-nodulating plants comprise a N<sub>2</sub>-fixing clade of the Rosid I lineage as assessed by phylogenetic analyses of the gene encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) (33, 35, 36). The identification of this clade has led to the suggestion that plants of this lineage are genetically predisposed to form N<sub>2</sub>-fixing root nodules (33); it also has raised questions about the specificity between actinorhizal plants and *Frankia* microsymbionts and the degree to which actinorhizal plants and their microsymbionts may have coevolved. Coevolution can be viewed as a “stepwise reciprocal selective response” (14), in which evolutionary changes in one symbiont have selected for compensatory evolutionary changes in the other symbiont. Analyses of 16S ribosomal DNA (rDNA) sequences have shown that *Frankia* sp. strains generally cluster in host plant-specificity groups, yielding the impression that the symbionts have coevolved to a degree (4, 12, 19, 26). If so, then the path and extent of coevolution are not straightforward. For example, some *Frankia* strains infect plants from families other than the original host plant family (3, 20, 39), and some microsymbionts in root nodules have 16S

rDNA signatures that belong to strains considered to infect plant families from outside the expected family (22a, 29).

Several 16S rDNA sequences of *Frankia* isolated from nodules of members of the family Elaeagnaceae are available, but, except for *Ceanothus* sp. (22, 30), members of the family Rhamnaceae have been neglected. Repeated attempts to isolate infective and effective *Frankia* strains from *Ceanothus* sp. root nodules have failed, leading to the notion that such *Frankia* strains are noncultivable or require unidentified growth factors or specialized growth conditions (3). Many nodulated members of the family Rhamnaceae are found only in South America, especially in Chile, and are confined to the semiarid region of central Chile as part of the xerophytic matorral (32). These plants have yielded *Frankia* strains quite readily, suggesting that different groups of *Frankia* may predominate in different genera of rhamnaceous plants (7–11).

To determine the diversity and relationships among *Frankia* that infect members of the family Rhamnaceae, we isolated and sequenced partial and full-length 16S rDNAs from *Frankia* microsymbionts living in root nodules of members of the families Elaeagnaceae and Rhamnaceae, particularly those from Chile. In addition, 16S rDNA sequences were obtained from cultures of *Frankia* isolated from root nodules of rhamnaceous plants. These sequences were compared to published *Frankia* 16S rDNA sequences in a phylogenetic analysis.

Table 1 lists the sources of root nodules and strains used in this study. Root nodules were obtained from plants, washed, and suspended in 95% ethanol. Nodules were stored at –20°C until they were processed for DNA isolation.

The procedures used for dissecting nodules, isolating DNA, and obtaining PCR products have been described previously (4). Nodules were rinsed thoroughly on a nylon mesh with a stream of cold tap water. Single lobes were dissected while being viewed with a dissecting microscope in 0.5 ml of sterile, freshly made TEA buffer (10 mM Tris-HCl, 1 mM Na<sub>2</sub> EDTA, 20 mM ascorbic acid [pH 7.6]) plus solid polyvinylpyrrolidone (PVPP [10 mg/ml]). Once the lobe periderm had been

\* Corresponding author. Mailing address: Department of Molecular and Cell Biology, U-44, University of Connecticut, Storrs, CT 06269-3044. Phone: (860) 486-4258. Fax: (860) 486-1784. E-mail: dbenson@uconnvm.uconn.edu.

TABLE 1. Sources of sequences used in this study

Source of nodule or <i>Frankia</i> strain	Location	GenBank accession no.	Source or reference
<b>Elaeagnaceae</b>			
<i>Elaeagnus angustifolia</i> isolate Ea1-2	Ecully, France	L40618	16
<i>Elaeagnus angustifolia</i> nod <sup>a</sup>	Willington, Conn.	AF064144	This study
<i>Elaeagnus</i> sp. nod	University of Chile, Santiago (nursery)	AF064145	This study
<i>Elaeagnus pungens</i> nod	Hamilton, New Zealand	U82099	12
<i>Shepherdia canadensis</i> isolate SCN10a	Cap-aux Oies, Quebec, Canada	L40619	21
<i>Hippophae rhamnoides</i> isolate HR27-14	Foncouverte, France	L40617	16
<b>Rhamnaceae</b>			
<i>Ceanothus americanus</i> nod	Willington, Conn.	AF063639	This study
<i>Ceanothus griseus</i> nod	Palmerston North, New Zealand (greenhouse)	U54608	4
<i>Colletia hystrix</i> nod	El Romeral, Santiago, Chile	AF063640	This study
<i>Colletia hystrix</i> isolate ChI5	Caleu, Santiago, Chile	AF064148	This study
<i>Discaria toumatou</i> nod	Canterbury, New Zealand	U54609	4
<i>Discaria serratifolia</i> isolate Ds12B	Conception, Chile	AF064149	This study
<i>Discaria trinervis</i> isolate DtI2	Bariloche, Argentina	AF064146	This study
<i>Retanilla ephedra</i> isolate ReI6	Cuesta La Dormida, Santiago, Chile	AF064150	This study
<i>Talguenea quinquenervia</i> nod	Santiago, Chile	AF063643	This study
<i>Talguenea quinquenervia</i> isolate TqI15	Rio Clarillo, Santiago, Chile	AF064147	This study
<i>Trevoa trinervis</i> nod ( <i>n</i> = 2)	Santiago, Chile	AF063644	This study
<i>Trevoa trinervis</i> nod	Santiago, Chile	AF063642	This study
<i>Trevoa trinervis</i> isolate TtI1	Las Vertientes, Santiago, Chile	AF064151	This study
<b>Betulaceae</b>			
<i>Alnus rubra</i> isolate ArI4	United States	L11307	Unpublished
<i>Alnus cordata</i> isolate AcoN24d	Canada	L40610	26
<i>Alnus rugosa</i> isolate ARgP5	Quebec City, Canada	L40612	26
<i>Alnus crispa</i> isolate AcN14a	Tadoussac, Canada	M88466	18
<i>Alnus rugosa</i> Sp <sup>+</sup> nod	Trois-Rivieres, Canada	L40956	26
Casuarinaceae; <i>Casuarina equisetifolia</i> isolate CeD	Senegal	M55343	25
Myricaceae; <i>Myrica nagi</i> nod	Lawjinrew, Meghalaya, India	L40622	26
Coriariaceae; <i>Coriaria arborea</i> nod	Mt. Tarawera, New Zealand	AF063641	This study
<b>Rosaceae</b>			
<i>Purshia tridentata</i> nod	Palmerston North, New Zealand (greenhouse)	U54611	4
<i>Dryas drummondii</i> nod	Gaspé, Canada	L40616	26
<b>Other</b>			
<i>Purshia tridentata</i> isolate PtI1	Wyoming	L41048	26
<i>Ceanothus caeruleus</i> isolate Cea1.3	Santo Tomás Atzinco, Mexico	U72717	30
<i>Ceanothus caeruleus</i> isolate Cea5.1	Santo Tomás Atzinco, Mexico	U72718	30
<i>Streptomyces ambofaciens</i>		M27245	28

<sup>a</sup> nod, obtained from a nodule.

removed, the nodule was rinsed in two additional 0.5-ml drops of TEA buffer with PVPP. The nodule was placed in a third drop of TEA buffer with PVPP, and the lobes were macerated with a sterile scalpel and forceps. Vesicle clusters were collected, and DNA was extracted as described previously by Benson et al. (4).

*Frankia* strains in culture were stored as mycelia suspended in 95% ethanol at -20°C. A loopful of the mycelia was suspended in 100 µl of Tris-EDTA and 100 µl of 0.2 N NaOH-1% (wt/vol) sodium dodecyl sulfate. The suspension was boiled, and DNA was precipitated in a manner identical to that described above.

*Frankia* 16S rDNA was amplified by PCR in 150-µl reaction mixtures consisting of the following: 12 µl of DNA template; 0.2 µM universal primer fD1 (40); 0.2 µM primer rDB1 (4); 200 µM (each) dATP, dCTP, dGTP, and dTTP; 2.5 mM MgCl<sub>2</sub>; 3.75 U of AmpliTaq DNA polymerase (Perkin-Elmer

Corp., Norwalk, Conn.); 100 µM sterile bovine albumin (Sigma Chemical Company, St. Louis, Mo.); 10 mM Tris-HCl (pH 8.3); and 50 mM KCl. The primers fD1 and rDB1 flank positions 28 to 419 on the *Escherichia coli* 16S rDNA and avoid amplification of plastid 16S rDNA that usually is present in the microsymbiont suspensions (4).

The PCR was done in a Perkin-Elmer model 2400 thermal cycler under the following conditions: primary denaturation at 95°C for two min, 35 cycles of denaturation at 94°C for 30 s, primer annealing at 57°C for 30 s, and extension at 72°C for 1 min. After 35 cycles, the reaction mixtures were held at 72°C for 7 min, and the thermal cycling was terminated. Following the PCR, the samples were centrifuged for 5 min to pellet precipitated albumin, and the supernatant was placed in a new microcentrifuge tube. Ten-microliter portions of the PCR products were analyzed on a horizontal 1.5% (wt/vol) FMC (Rockland, Maine) agarose gel to check for the presence of a

single band corresponding to the expected size of the product. The amplicons were purified on a Qiagen (Chatsworth, Calif.) column (PCR purification kit) according to the manufacturer's protocol. The DNA in the eluted samples was quantified with a DyNA Quant 200 DNA fluorometer (Hoefer Pharmacia Biotech, Inc., San Francisco, Calif.).

Full-length microsymbiont 16S rDNA amplicons for *Ceanothus americanus*, *Colletia hystrix*, *Coriaria arborea*, and *Trevoa trinervis* were obtained by PCR in a final volume of 50  $\mu$ l with 4  $\mu$ l of *Frankia* DNA serving as template under the conditions described above. For this reaction, the universal primers fD1 and rD1 were used to amplify almost the entire 16S rDNA gene (40). To remove plastid DNA, 16  $\mu$ l of the amplified *Frankia*-plastid 16S rDNA reaction mixture was digested for 1 h at 37°C with *Pvu*II, which cleaves plastid but not *Frankia* 16S rDNA. Two microliters of this reaction mixture was then used as a template (in triplicate) for a second series of three 50- $\mu$ l PCRs with the primers fD1 and rD1 (40). The three completed PCRs were combined, the mixtures were centrifuged for 5 min at 13,500  $\times$  g, and the supernatant was saved. The DNA was purified with a Qiagen column and quantitated.

The purified amplicons were cycle sequenced and analyzed with an Applied Biosystems (ABI) Prizm sequencer (Perkin-Elmer) with an ABI cycle sequencing kit (Perkin-Elmer) with 2 pmol of primer and 100 fmol (ratio of 20:1) of double-stranded DNA. For short amplicons, sequencing was conducted in both directions with primers binding in the middle of the template and at both ends. For the longer *Frankia* 16S rDNA amplified from root nodules, sequencing was done in both directions with primers spaced about every 300 bp.

Sequences were aligned by using the Genetics Computer Group Pileup program and ClustalW (13, 37). Phylogenetic trees were constructed by using the programs in PHYLIP (neighbor joining) (15) and PUZZLE (maximum likelihood) (34). The trees were drawn with the program TREEVIEW (27).

To minimize the sequencing necessary to place nodule microsymbionts in a *Frankia* strain group, we amplified a portion of the 16S rDNA gene that provided a balance between sequence variability and length. Preliminary work with published and unpublished sequences showed that the 378-bp region proximal to the 5' terminus (corresponding to *E. coli* positions 28 to 419, between primers) had about 45% of the total variable sites among *Frankia* strains, with the remainder found in the 1,100-bp 3' fragment. We reasoned that if sequences from different nodules were identical in the short region, then differences in the remainder of the molecule would be unlikely to place an unknown strain in another clade. This approach was confirmed by using nearly full-length amplicons from some of the same nodules.

We obtained a total of 15 sequences. They were amplified from six nodules and six isolated strains of *Frankia* from six of the eight actinorhizal genera in the family Rhamnaceae, two nodules from *Elaeagnus* sp. (Elaeagnaceae), and one nodule from *Coriaria arborea* (Coriariaceae). Published sequences used for the phylogenetic analysis were from strains that infect each genus in the family Elaeagnaceae (*Elaeagnus*, *Hippophaë*, and *Shepherdia*) and from other *Frankia* strains. An alignment of *Frankia* 16S rDNAs showed that with the exception of microsymbionts from *Ceanothus americanus* and *Ceanothus griseus*, all microsymbionts and isolates examined from the families Elaeagnaceae and Rhamnaceae had a signature 2-base deletion at positions 50 and 51 (between primers) compared to other *Frankia* sequences available (data not shown).

The overall diversity of *Frankia* strains that infect members of the families Elaeagnaceae and Rhamnaceae is likely to be low, since identical sequences were commonly obtained from

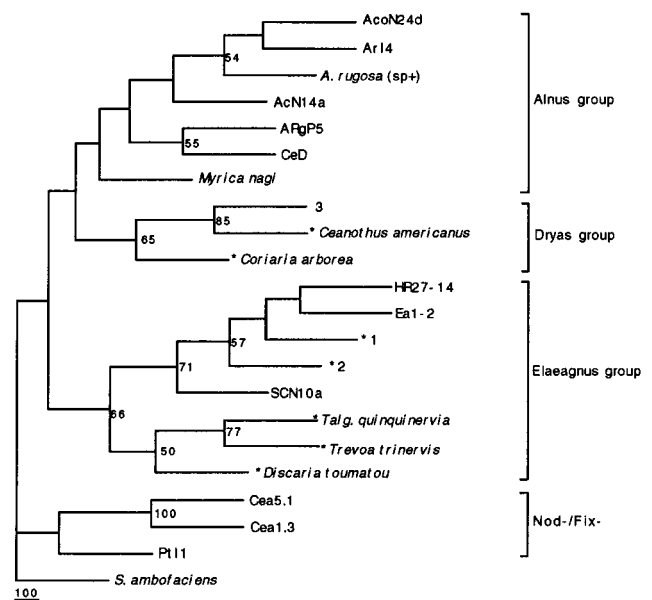


FIG. 1. Neighbor-joining phylogram for *Frankia* 16S rDNA. Sequences were aligned by using ClustalW. The aligned sequences were then analyzed by using PHYLIP. Asterisks denote sequences obtained in this study. Sequence 1 was found in the nodule inhabitants of *Elaeagnus angustifolia*, *Elaeagnus pungens*, an unidentified *Elaeagnus* species, and *Trevoa trinervis* (accession no. AF064144, U82099, AF064145, and AF063644, respectively) and from the *Frankia* sp. strain Dt12, Ea1-2, HR27-14, and TqI15 (accession no. AF064146, L40618, L40617, AF064147, respectively). Sequence 2 was from microsymbionts in a *Colletia hystrix* nodule and the isolates ChI5, Ds12B, ReI6, and TtI1 (accession no. AF063640, AF064148, AF064149, AF064150, and AF064151, respectively). Sequence 3 was found in the root nodule inhabitants from *Ceanothus griseus*, *Dryas drummondii*, and *Purshia tridentata* (accession no. U54608, L40616, and U54611, respectively). The *Streptomyces ambofaciens* sequence was used to root the tree. Bootstrap values are shown as percent of 400 replicates. The bootstrapped samples were used to generate 400 distance matrices by using DNADIST. The assumptions made to construct the distance matrices were those of a Kimura two-parameter model with transversions weighted twice as heavily as transitions. Neighbor-joining trees were then constructed from the distance matrices by using NEIGHBOR. A consensus tree was found by using CONSENSE and viewed with TREEVIEW.

nodules and strains. Sequence 1 (Fig. 1) is found in nodules from *Elaeagnus angustifolia* (accession no. AFO64144 in Table 1; Willington, Conn.), *E. pungens* (U82099; Hamilton, New Zealand), an *Elaeagnus* sp. growing in Chile (AF064145), and *Trevoa trinervis* (AF063644; Santiago, Chile). In cultured strains, sequence 1 is in Ea1-2 from *E. angustifolia* and HR27-14 from *Hippophaë rhamnoides*, both members of the family Elaeagnaceae that were originally isolated in France (16), and DtI2 from *Discaria trinervis* (Bariloche, Argentina) and TqI15 from *Talguenea quinquerivaria* (Río Clarillo, Chile), both members of the family Rhamnaceae (8).

Sequence 2 (Fig. 1), which differs from sequence 1 at only one position in the 378-bp fragment, is found in microsymbionts in nodules of *Colletia hystrix* (AFO63640; Santiago, Chile) and the isolated strains ChI5 from *C. hystrix* (Santiago), Ds12B from *Discaria serratifolia* (Concepcion, Chile), ReI6 from *Retanilla ephedra* (Santiago), and TtI1 from *T. trinervis* (Santiago). This lack of diversity is noteworthy in view of the wide geographic distribution of the nodules and strains analyzed. Low diversity in *Frankia* 16S rDNA sequences has previously been reported for lineages that infect *Coriaria* species (Coriariaceae) and members of the family Rosaceae plus *Ceanothus* (4, 12, 24).

Variability of 16S rDNA sequences among microsymbionts that infect plants from the families Elaeagnaceae and Rham-

naceae, excluding the microsymbionts of *Ceanothus americanus*, occurs at six sites within the 378-bp fragment. The sequences in microsymbionts from *C. americanus* root nodules are quite different; they differ at 15 sites from the collective sequences of strains or microsymbionts of all genera of the families Elaeagnaceae and Rhamnaceae examined and lack the 2-base deletion typical of sequences from strains infecting plants in these families.

To identify additional variable sites among strains in nodules from the Elaeagnaceae-Rhamnaceae group, we sequenced most of the 16S rRNA gene (1,395 nucleotides) from microsymbionts infecting *Ceanothus americanus*, *Colletia hystrix*, and *Trevoa trinervis*. The total variability among five widely collected sequences from the elaeagnus group of *Frankia*, including those from rhamnaceous plants, occurs at only 14 sites, with 6 sites (43%) located in the first 378 bp. The total variability of *C. americanus* with respect to all the elaeagnus sequences occurs at 34 sites, including the 2-bp deletion. As sequences from more nodules become available, additional variability will no doubt be observed, but based on the short and long sequences that have been reported here and elsewhere, the depth of the diversity within the elaeagnus group of *Frankia* seems likely to remain shallow.

The presence of identical *Frankia* 16S rDNA sequences in plants of the families Elaeagnaceae and Rhamnaceae supports the observation that species of *Elaeagnus* and *Hippophaë* (Elaeagnaceae) can be nodulated by isolates from *Colletia* sp. (Rhamnaceae) (17). Our findings also support the results of phylogenetic analyses of strains infective of *Elaeagnus* (isolated from genera of the families Elaeagnaceae and Rhamnaceae) that indicated monophyly among the isolates (23). The only exceptions found to monophyly in this group are the microsymbionts in *C. americanus* and *C. griseus*. Microsymbionts in these nodules appear to be related to *Frankia* sp. strains found so far only in rosaceous actinorhizal plants.

Six isolates used in this study (ChI5, Ds12B, DtI2, ReI6, TqI15, and TtI1) have been phenotypically characterized and differ in some traits, such as physiology, sporulation qualities, resistance to antibiotics, and others (6, 8, 9, 11). Despite having similar 16S rDNA sequences, strain ReI6 grows well on sucrose, whereas TtI1 grows better with pyruvate than with sucrose (6). Also, the strains exhibit some polymorphism in their electrophoretic patterns of esterases, diaphorase, and malate dehydrogenase (unpublished). Thus, strains having identical partial sequences may differ in minor physiological properties. A more precise resolution of strain diversity could be achieved by using a more rapidly evolving semantide for analysis.

A phylogenetic analysis of the 16S rDNAs was done by using sequences from the organisms listed in Table 1. Both neighbor-joining (PHYLIP) (15) and maximum likelihood (PUZZLE) (34) (not shown) were used to derive phylogenetic trees. Both methods clustered sequences from microsymbionts that nodulate plants in the families Elaeagnaceae and Rhamnaceae. However, sequences from *C. americanus* and *C. griseus* formed a separate clade together with strains that inhabit nodules from members of the families Coriariaceae and Rosaceae (Fig. 1). The topology of the tree is similar to those published in previous studies (4, 12, 19, 26). Consistent features of such trees include the clustering of strains from *Alnus* sp. (Betulaceae) with those from *Casuarina* sp. (Casuarinaceae) and *Myrica nagi* (Myricaceae) and the distant placement of strains PtI1, Cea5.1, and Cea1.3, all Nod<sup>-</sup> Fix<sup>-</sup> isolates that form a clade of *Frankia*-like actinomyces that are sometimes isolated from actinorhizal nodules. Cea5.1 and Cea1.3 were isolated as co-symbionts with an effective symbiont in *Ceanothus caeruleus* root nodules (30).

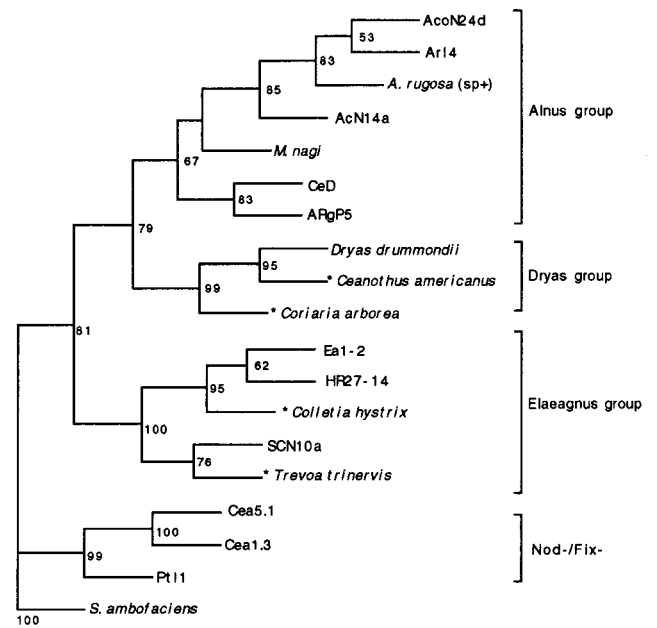


FIG. 2. Neighbor-joining cladogram for nearly full-length *Frankia* 16S rDNA. Sequences were aligned by using ClustalW. The aligned sequences were analyzed as described in the legend to Fig. 1.

To determine if longer 16S rDNA sequences would yield trees with a similar topology, we constructed trees by using published 16S rDNA sequences plus the nearly full-length sequences from the microsymbionts of *C. americanus*, *Colletia hystrix*, and *Trevoa trinervis*. In addition, we sequenced the 16S rDNA gene from *Coriaria arborea* (Coriariaceae). The resulting neighbor-joining tree (Fig. 2) has virtually the same topology as that shown in Fig. 1, with the exception that the bootstrap values are higher. The sequences from *T. trinervis* and *C. hystrix* again clustered with those from *Frankia* strains that infect members of the family Elaeagnaceae (*Elaeagnus*, *Hippophaë*, and *Shepherdia*). Also in agreement with Fig. 1, the nearest neighbor of the *C. americanus* microsymbiont was the sequence found in nodules of *Dryas drummondii* (Rosaceae) followed by the microsymbiont from *Coriaria arborea* (Coriariaceae).

A previous study using partial 16S rDNA sequences from a variable region different from those used here suggested that *Ceanothus* sp., like others from the family Rhamnaceae, select *Frankia* strains belonging to the elaeagnus group (22). To address this apparent conflict, we constructed phylogenetic trees (not shown) based on the sequences used by Murry et al. (22) plus the *C. americanus* sequence. In agreement with Murry et al., their sequences clustered closer to the elaeagnus *Frankia* strains than to the rosaceous *Frankia* strains (not shown). However, the *C. americanus* sequences still clustered with those from rosaceous plants. Another recent study that used full-length 16S rRNA genes amplified from root nodules of *Ceanothus caeruleus* showed that effective *Frankia* strains within those nodules had a short sequence that was identical to that found in *C. griseus* nodules and a long sequence having only four differences from the inhabitant of *Dryas drummondii* (Rosaceae) root nodules (30). The overall diversity of *Frankia* strains that inhabit *Ceanothus* nodules is therefore not clear at present, because different studies have analyzed different species. To resolve this issue, a more in-depth study of more species within this one genus is necessary.

The close relationship between *Frankia* strains that infect

most plants belonging to the families Elaeagnaceae and Rhamnaceae suggests that the last common ancestor of both families was nodulated by *Frankia* strains similar to those currently occupying the nodules. Actinorhizal plants from both families comprise a monophyletic lineage within the N<sub>2</sub>-fixing clade (based on *rbcL* sequences) (33, 35, 36) and have similar fossil pollen records. Both first appear in the Oligocene epoch of the Tertiary period (about 31 million years ago). The low diversity of *Frankia* strains in these nodules, compared to that of strains infecting plants from the families Betulaceae and Myricaceae, for example, may reflect the relatively recent divergence of this group of actinorhizal plants.

Although plants of the families Elaeagnaceae and Rhamnaceae permit infection primarily by a defined group of *Frankia* strains, the elaeagnus group, strong conclusions about the degree to which these plants restrict the entry of other strains that normally infect other plants must await a more extensive sampling of individual species. The importance of conducting such studies is underscored by reports showing that plants such as *E. angustifolia* can be quite promiscuous in accepting *Frankia* strains when tested in the laboratory (1). The degree to which such promiscuity occurs in the field is unknown.

From the perspective of the microsymbiont, one cannot conclude that the elaeagnus group of *Frankia* strains specialize in infecting only plants in the families Rhamnaceae and Elaeagnaceae. In fact, *Frankia* strains from the elaeagnus group can nodulate some plants in the family Casuarinaceae, in particular, *Gymnostoma* sp., in which elaeagnus strains appear to be the primary microsymbionts (22a). Elaeagnus strains have also been shown to infect members of the families Betulaceae (5) and Myricaceae (39) in the laboratory. The situation in the field, in which competitive interactions are more intense, has not been addressed.

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