APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1989, p. 2161–2166
0099-2240/89/092161-06$02.00/0

Vol. 55, No. 9

Physiological, Chemical, Morphological, and Plant Infectivity Characteristics of *Frankia* Isolates from *Myrica pensylvanica*: Correlation to DNA Restriction Patterns†

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Received 3 February 1989/Accepted 12 June 1989

The filter exclusion method was used to isolate *Frankia* strains from *Myrica pensylvanica* (bayberry) root nodules collected at diverse sites in New Jersey. A total of 16 isolates from five locations were cultured. The isolates were characterized by morphological, chemical, physiological, and plant infectivity criteria and compared with genomic DNA restriction pattern data, which were used to assign the isolates into gel groups (see accompanying paper). The isolates from *M. pensylvanica* evaluated in this study were characteristic of *Frankia* physiological group B strains and were indistinguishable on the basis of whole-cell wall chemistry and diaminopimelic acid isomer analysis. Distinct differences in the spectrum of utilized organic acids and carbohydrates were observed among the isolates and were the only phenotypic criteria by which the isolates could be separated and assigned into separate groups. In general, isolates within a restriction pattern gel group had identical utilization patterns, whereas intragroup isolates had different utilization patterns. Correlation of these phenotypic characteristics with the results of molecular analysis revealed an exclusive carbohydrate and organic acid utilization pattern for each gel group as established by restriction pattern analysis.

Actinomycetes of the genus *Frankia* form nitrogen-fixing root nodules on a diverse group of dicotyledons and are important contributors of fixed nitrogen. From studies with a variety of *Frankia* strains, it is apparent that there is a large degree of morphological and chemical uniformity among isolates and that most can be classed into one of two groups on the basis of serology, physiology, and cross-inoculation studies (17a). The identification of individual strains is a complex undertaking and has typically relied on detailed morphological and biochemical studies. At present, no species delineations are agreed upon, although the genus *Frankia* can be well defined.

The present study is part of a larger investigation conducted to assess diversity among *Frankia* isolates from *Myrica pensylvanica* (bayberry) nodules collected in various locales in New Jersey. A concurrent study of *Frankia* isolates from *M. pensylvanica* has demonstrated the utility of DNA restriction patterns as a means to differentiate quickly between morphologically similar isolates (8). Sixteen isolates were divided into nine separate gel groups. Each isolate had a distinctive reproducible fingerprint which was not altered by repeated DNA extractions or culture conditions (8). However, the validity of using DNA restriction patterns to group *Frankia* isolates has not been confirmed by more-traditional taxonomic criteria. Since no criteria have yet been established for designating a *Frankia* species, it is not possible at present to correlate restriction patterns and species.

The objectives of the present study were twofold: (i) to identify and characterize *Frankia* isolates by morphological, chemical, physiological, and plant infectivity criteria, and (ii) to determine how isolate groups distinguished by the above criteria are related to gel groups established from restriction pattern data. This information is necessary to test the validity of DNA restriction patterns as a tool for the separation and classification of individual frankiae. The isolates used in this study have been previously arranged into gel groups on the basis of restriction patterns (8).

MATERIALS AND METHODS

Collection site descriptions. Nodules were collected from *M. pensylvanica* Loisel (bayberry) plants located at the following sites: I, Green Knoll, north of intersection of N.J. Route 22 and Interstate 287, Somerset County, N.J., 40°34′ N, 74°36′ W; II, Princeton Nurseries, Princeton, Mercer County, N.J., 40°20′ N, 74°39′ W; III, Harvey Cedars, Long Beach Island, Ocean County, N.J., 39°41′ N, 74°09′ W; IV, Island Beach State Park, Ocean County, N.J., 39°52′ N, 74°05′ W; and V, Tinton Falls landfill, Tinton Falls, Monmouth County, N.J., 40°18′ N, 74°05′ W.

Nodule collection and *Frankia* isolation. *M. pensylvanica* plants were identified by standard botanical keying methods and by comparison with plants at the Chrysler Herbarium, Rutgers University. Intact, light-colored nodules were selected. Nodules from separate plants in proximity to each other were collected.

Nodules were stored on ice during transit to the laboratory and were processed on the same day that they were collected. After removal of loose soil, the nodules were placed in a fiber mesh bag and washed under a stream of water. The nodules were surface sterilized with 1.05% sodium hypochlorite for 10 min with gentle shaking, followed by several rinses in 0.1 M sodium bicarbonate. After an overnight incubation at 25°C in sterile water, the nodules were resterilized for 10 min, rinsed in distilled water, and treated for the isolation of *Frankia* strains by the methods of Benson

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† New Jersey Agricultural Experiment Station publication no. D-15288-2-89.

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(6). The nodules were homogenized aseptically in 5 ml of water in 15-ml Ten Broeck tissue homogenizers to release the endophyte vesicle clusters. The homogenate was filtered through a double-mesh filtration system consisting of an upper mesh of 50 μm and a lower mesh of 20 μm. The 50-μm screen retained large particles, allowing vesicle clusters to pass through. The vesicle clusters which were retained on the 20-μm mesh were isolated by the pour plate method on Benson minimal medium (6) containing 400 μg of cycloheximide per liter and 15 μg of nystatin per liter in petri dishes (100 by 15 mm). Microscopic examination of the plates ensured that developing hyphae were originating from vesicle clusters. *Frankia* colonies approximately 2 to 3 mm in diameter were removed from the agar and macerated in 1 ml of sterile distilled water. Samples (0.1 ml) of the macerated colony were dispensed into duplicate tubes of eight different media (L/2, L+S_2 [14]; S, S+Tw [13]; B/2 [per liter of distilled water: 0.5 g of yeast extract, 0.5 g of beef extract, 1.0 g of N-Z amine type A, 5.0 g of glucose], defined liquid medium [20], nitrogen-free defined propionate medium [DPM] [3], and DPM/N [DPM plus 8 mM NH_4Cl]) (20) and incubated at 27°C without agitation in loosely capped test tubes.

Frankia isolates were subcultured at 4-month intervals into the medium which provided the best growth and incubated at 27°C without agitation. The cultures were agitated once weekly for 5 min at 100 rpm. Hyphal mats were broken during each subculturing procedure by passing the mats through thin-bore 1-ml pipettes.

Vesicle formation was induced by subculturing into DPM (3). Isolated colonies were harvested by centrifugation and washed twice with DPM and then inoculated into 5 ml of DPM in loosely capped 20-ml test tubes. Vesicle formation and colony morphology were observed microscopically. $NZ_{D+S}P$ is NZ_D (14) plus S medium salts (1.0 ml/liter) plus propionate (0.5%, wt/vol).

For carbohydrate and amino acid utilization, 4-week-old cultures were harvested by centrifugation and washed twice with sterile distilled water. The washed cells were homogenized in Ten Broeck tissue homogenizers, and 0.1-ml samples were used to inoculate 15-ml test tubes containing 5 ml of the appropriate medium (14).

Whole-cell analysis was performed by the method of Lechevalier and Lechevalier (16).

Plant cultivation, culture, and inoculation. Seeds were collected from a single mature *M. pensylvanica* plant and cold stratified in the dark at 4°C for 16 weeks in sterile moist 50:50 mixtures of perlite and peat moss. Plants were cultivated in 275-ml glass jars containing 250 ml of one-quarter-strength Hoagland nutrient solution, pH 6.0 (10). Plants were grown in a controlled environment cabinet at 25°C and a 400-microeinstein light intensity. A 16-h photoperiod was maintained. One-quarter-strength Hoagland nitrogen-free solution was substituted 2 weeks prior to *Frankia* inoculations and maintained for the duration of the experiment.

Four-week-old seedlings were inoculated with first-subculture growth of *Frankia* isolates. Owing to the limited number of plants available, only two to three plants were inoculated with each *Frankia* isolate. Seedlings were inoculated with a packed cell volume equivalent to 0.02 ml by a modification of the methods of Lalonde and Calvert (11). *Frankia* mycelia were washed in distilled water and disrupted with a Ten Broeck tissue homogenizer to give a suspension of filaments. The inoculum suspension was carefully spread onto the total plant root. Growth solution volume was restored daily. The nitrogen-free Hoagland solution was changed weekly after nodules appeared. Uninoculated seedlings served as controls.

Acetylene reduction. Ten weeks after nodule development, nitrogenase activity was measured by the acetylene reduction technique (9, 21). Root nodules were incubated immediately after excision in an atmosphere containing 10% (vol/vol) acetylene (Matheson Gas Co., East Rutherford, N.J.) in 20-ml glass vials with serum stoppers. Samples were incubated at 27°C for 1 h. Acetylene and ethylene were analyzed on a Perkin-Elmer (Sigma 4) gas chromatograph equipped with an H_2-air flame ionization detector and a column packed with Porapak N (192 by 0.3 cm). The column was operated at 100°C. N_2 was used as the carrier gas at a flow rate of 30 ml/min.

RESULTS

Isolation and colony morphology. Owing to the slow growth rate of *Frankia* spp. compared with those of other soil microorganisms, pretreatment of the nodules and specialized plating conditions were necessary to minimize contamination. Intact, healthy nodules, NaOCl as a nodule-sterilizing agent, cycloheximide and nystatin in the growth medium, mineral salts agar, and pour plate methods helped reduce bacterial and fungal contamination. The double-mesh filtration system provided an inoculum enriched in *Frankia* vesicle clusters and minimized the amount of nodule tissue and plant debris present in the preparation. These treatments provided for the extended incubation periods required for *Frankia* colony development.

Criteria for the identification of frankiae on pour plates included slow, microaerophilic growth (growth position in test tube), the emergence from plant vesicles of branched hyphae approximately 1 to 2 μm in diameter, and the production of sporangia with numerous spores measuring 1 to 3 μm in diameter. Differences were noted in the degree of hyphal branching, the growth rate, and the compactness of the colonies. The isolates developed a hyaline mycelium on agar plates.

These morphological characteristics were sufficient for tentative isolate identification. Minimum time for the appearance of individual hyphae emerging from vesicle clusters was 1 to 2 weeks. For most isolates, an incubation period of 1 to 2 months was required for colonies to reach sufficient size (2 to 3 mm) to permit transfer to liquid medium.

The best growth medium was determined by subculturing single *Frankia* isolates into a variety of media (L+S_2, L/2, S+Tw, B/2, DPM, and DPM/N). In general, L/2, S+Tw, and DPM/N media provided the most vigorous growth. The isolated strains are listed in Table 1.

Morphological, chemical, and physiological characteristics. To compare morphological characteristics, we cultured all isolates in L/2 medium. All isolates except RBR162001 and RBR162003 of gel group C grew to some extent in L/2. Isolates RBR162001 and RBR162003 developed well in DPM/N. The *Frankia* isolates were similar to others of the *Alnus-Myrica* grouping (Table 2). The isolates displayed white mycelia in L/2 submerged cultures and in a variety of other growth media. Branching hyphae fell into two major groups based on diameter, 0.5 to 1.0 and 0.8 to 1.5 μm, with single isolates having a range of hyphal diameters. The degree of sporangium formation varied with the medium utilized. In L/2, no sporangia were evident for any of the gel group A isolates, whereas two of the four gel group B isolates formed sporangia. Isolates of groups D and E formed sporangia in L/2. Isolate RBR162018 formed brown-

TABLE 1. Catalog of *Frankia* strains isolated from root nodules of *M. pensylvanica* Loisel

Site	Location	Isolate no.[a]
I	N.J. Route 22 and Interstate 287, Green Knoll	RBR162001 RBR162002 RBR162003
II	Princeton Nursery	RBR162005
III	Harvey Cedars, Long Beach Island	RBR162008 RBR162009 RBR162010 RBR162011 RBR162012 RBR162013 RBR162014
IV	Island Beach State Park	RBR162017 RBR162018 RBR162019
V	Tinton Falls	RBR162020 RBR162021

[a] RBR, Raanan Bloom, Rutgers University; 16, *Myrica*; 20, *pensylvania*; 01 to 21, isolate number (12).

black spores in DPM, and group C isolates formed sporangia in DPM/N. On medium NZ_{D+S}, the following isolates formed sporangia: one of the group A isolates (RBR162014) and isolates of groups C, E, G, H, and I. In L/2 and NZ_{D+S}, the sporangia were concolorous with the hyphae. Distinctive differences in spore size were not evident. Vesicles were not apparent for any of the isolates on complex N-rich medium but were expressed after an incubation period of 2 to 3 weeks in DPM. Various degrees of vesicle formation were apparent, with group A isolates demonstrating little or no vesicle development.

A soluble yellow pigment was produced by isolate RBR162005, whereas a green soluble pigment formed in RBR162018 cultures. None of the other isolates produced any pigments. All the isolates grew under microaerophilic conditions. They developed best in submerged cultures and did not grow on the surface of spread plates.

Identical diagnostic sugar patterns and isomers of diaminopimelic acid were found for all the isolates. All contained the following whole-cell sugars: galactose, glucose, mannose, xylose, ribose, and rhamnose plus an unidentified hexose similar in mobility and reaction to a sugar tentatively identified as 2-*O*-methyl-D-mannose by Mort et al. (19). The presence of xylose and the absence of arabinose is typical of *Frankia* strains having type D actinomycetal sugar patterns. The cell wall composition of the isolates examined was characterized by the presence of *meso*-diaminopimelic acid. Of the strains analyzed, two of the four group B isolates (RBR162017 and RBR162019) also contained faint amounts of an unknown polar amino acid, which gave a blue reaction with ninhydrin (17a). The sugar patterns and diaminopimelic acid isomer confirmed these *Frankia* isolates as having a type III cell wall, typical for the genus.

Carbohydrate and organic acid source utilization pattern experiments were conducted (Table 3). A period of 4 to 12 weeks was required before growth could be established on some of the substrates. The experiment was conducted in duplicate and evaluated after 8 and 16 weeks of incubation. No growth was observed for any of the isolates in the control medium NZ_{D+S}. Significant differences in carbohydrate uti-

TABLE 2. Morphological characteristics of *Frankia* isolates[a]

Isolate	Gel group	Soluble pigment	Vegetative hyphae		Spore formation		Vesicle	
			Color	Size[b]	L/2	$NZ_{D+S}P$	Formation	Size[c]
RBR162008	A	None	White	A	−	−	+	C
RBR162010	A	None	White	A	−	−	+	C
RBR162013	A	None	White	A	−	−	−	−
RBR162014	A	None	White	A	−	+	+	C
RBR162011	B	None	White	B	+	−	+	D
RBR162012	B	None	White	A	−	−	ND[d]	ND
RBR162017	B	None	White	A	−	−	+	D
RBR162019	B	None	White	A	+	−	+	D
RBR162001	C	None	White	A	+[e]	+	+	D
RBR162003	C	None	White	A	+[e]	ND	+	D
RBR162002	D	None	White	B	+	−	+	C
RBR162005	E	Yellow	White	B	+	+	+	C
RBR162009	F	None	White	A	−	−	+	D
RBR162018	G	Green	White	A	−	+	+	D
RBR162020	H	None	White	A	−	+	+	D
RBR162021	I	None	White	A	−	+	+	C

[a] All isolates were cultured in L/2 medium unless otherwise noted. Vesicle formation was determined in DPM.
[b] A, 0.8 to 1.2 μm; B, 0.5 to 1.0 μm.
[c] C, 1.5 to 3.0 μm; D, 2.0 to 4.0 μm.
[d] ND, Not determined.
[e] Cultured in DPM/N.

TABLE 3. Physiological tests of *Frankia isolates*

Isolate	Gel group	Carbohydrate and organic acid utilization at 0.5%[a]											
		NZ_{D+S}	A_2	G_2	M_1	R_2	T	X_1	Ac	Fu	Pr	Py	Su
RBR162008	A	−	−	−	−	−	−	−	−	−	+	−	−
RBR162010	A	−	−	−	−	−	+[b]	−	−	−	+	−	−
RBR162013	A	−	−	−	−	−	+[b]	−	−	−	+	−	−
RBR162014	A	−	−	−	−	−	+[b]	−	−	−	+	+	−
RBR162011	B	−	−	−	+	−	+[c]	−	+	−	+	+	−
RBR162012	B	−	−	−	+	−	+[c]	−	+	−	+	+	−
RBR162017	B	−	−	−	+	−	+[c]	−	+	−	+	+	−
RBR162019	B	−	−	−	+	−	+[c]	−	+	−	+	+	−
RBR162001	C	−	−	−	−	−	−	−	−	−	+	−	−
RBR162003	C	−	−	−	−	−	−	−	−	−	+	−	−
RBR162002	D	−	−	−	+	−	+	−	−	−	+	+	−
RBR162005	E	−	−	−	−	−	−	−	+	−	+	−	−
RBR162009	F	−	−	−	+	−	+[c]	−	+	−	+	−	−
RBR162018	G	−	−	−	−	−	+[b]	−	+	−	+	+	−
RBR162020	H	−	−	−	−	+	−	−	−	−	+	−	−
RBR162021	I	−	−	−	−	+	−	−	−	−	+	+	−

[a] A_2, Arabinose; G_2, glucose; M_1, maltose; R_2, rhamnose; T, trehalose; X_1, xylose; Ac, acetate; Fu, fumarate; Pr, propionate; Py, pyruvate; Su, succinate; +, utilized, growth increased over that of basal medium (NZ_{D+S}); −, not utilized. Readings made after 3 months of incubation at 28°C.
[b] Green-brown soluble pigment.
[c] Yellow soluble pigment.

TABLE 4. Inoculation of *M. pensylvanica* plants[a] with *Frankia* strains

Isolate	Gel group	Nodulation[b]	Avg plant wt (g [fresh wt])	Acetylene reduction C_2H_4/g of nodule per h; (nmol of $\bar{x}$ ± SD)
RBR162008	A	2/2	14.84	124.5 ± 26.98
RBR162010	A	2/2	19.26	222.8 ± 103.9
RBR162013	A	2/2	16.77	146.8 ± 56.18
RBR162014	A	2/2	14.97	244.0 ± 107.8
RBR162011	B	2/2	12.11	269.6 ± 193.3
RBR162012	B	2/2	13.17	198.0 ± 51.54
RBR162017	B	2/2	10.29	131.8 ± 66.66
RBR162019	B	2/2	18.00	106.0 ± 30.41
RBR162003	C	1/1	17.14	ND[c]
RBR162002	D	2/2	12.79	256.1 ± 72.77
RBR162005	E	2/2	11.01	478.6 ± 69.32
RBR162009	F	2/2	15.92	312.6 ± 81.25
RBR162018	G	2/2	13.39/6.82[d]	ND
RBR162020	H	1/2	15.93/6.08[d]	198.0 ± 12.00
RBR162021	I	2/2	18.75	188.5 ± 57.64

[a] Plants harvested 14 weeks after inoculation.
[b] Number of plants nodulated/number of plants inoculated.
[c] ND, No data. Effectivity determined by reversal of nitrogen deficiency symptoms.
[d] Delayed nodulation and poor development exhibited by second plant.

lization patterns were observed between the isolates and gel groups, with minor variations for the isolates within a single gel group.

Growth rates on most substrates were slow. The best growth occurred for isolates on the basal medium plus trehalose. Growth was poor on maltose and rhamnose. Soluble pigment production was apparent with most of the isolates during growth on trehalose. All isolates in gel group B and the single isolates of groups D and F utilized maltose. Rhamnose was metabolized by group H and I isolates, whereas trehalose supported growth of three of four isolates from group A, group B isolates, and single isolates of groups D, F, and G. All the strains grew on propionate, whereas succinate and fumarate were not utilized. Acetate was utilized by the four group B isolates and the single isolates of groups E, F, and G. Growth on pyruvate occurred with only one of the four group A isolates and with isolates of groups D, G, and I.

The spectrum of compounds metabolized for growth by isolates within a gel group remained constant except for the above-mentioned minor exceptions. The utilization patterns varied between groups, and no two groups demonstrated the same pattern when evaluated as a whole. Individual isolates in separate groups did show similar spectra (for example, isolate RBR162008 and group C isolates). Group B isolates were most versatile, being capable of utilizing five substrates. Group C isolates were the least versatile, only growing on trehalose and propionate. Most of the isolates utilized a minimum of two carbon sources.

Infectivity and effectivity characteristics. The infectivity and effectivity characteristics of the isolates were evaluated (Table 4). Effectivity was determined by measuring acetylene reduction rates and/or by visual observation of plant growth following nitrogen deprivation and the appearance of nodules. A maximum of two plants per isolate were available for these trials owing to the difficulty associated with germination and handling of *M. pensylvanica* seedlings.

Nodulation took place within 15 to 22 days for most isolates, with partial reversal of plant nitrogen deficiency systems appearing 1 week after the development of nodules. For all isolates except RBR162018 and RBR162020, both plants developed nodules within a short period of each other. With isolates RBR162018 and RBR162020, delayed nodulation was observed for one of the two plants, with delays of 30 and 39 days, respectively. With the exception of those plants with delayed nodulation, an average of 16.02 g of biomass was produced by the nodulated plants over the 16-week growth period. Although acetylene reduction rates were quite variable, all the isolates tested were infective and effective.

DISCUSSION

The primary objective of this study was to evaluate strain diversity among *Frankia* actinomycetes isolated from the actinorhizal plant *M. pensylvanica* (bayberry). Few *M. pensylvanica* isolates have been cultured, and only isolates LLR162001 and DDB16201010 have been used for any extensive studies (2, 15). Thus, the isolates investigated in this study increase our understanding of the symbionts of bayberry. To accomplish this objective, *Frankia* strains were isolated from root nodules collected at diverse sites in New Jersey and evaluated by both phenotypic and molecular methods.

The results of this study indicated that most classical identification methods for *Frankia* isolates lack the resolution required to separate the isolates into subgroups other than physiological groups A and B. Whereas restriction pattern data clearly divided the 16 isolates into nine separate gel groups (8), morphological, chemical, and plant infectivity data provided no clear basis for assigning isolates to specific groups.

Distinct differences in the spectrum of utilized carbohydrates and organic acids were, however, observed among the isolates. Thus, this characteristic provided the only phenotypic criterion by which the isolates could be assigned to separate groups. Correlation of these phenotypic characteristics with the results of molecular analyses (8) revealed an exclusive carbohydrate and organic acid utilization pattern for each gel group as established by restriction pattern data. With few exceptions, isolates within a gel group had identical utilization patterns, whereas intergroup isolates had different utilization patterns. The data demonstrate that isolates with similar restriction patterns shared additional characteristics by which they could be differentiated from isolates indistinguishable in morphology, chemistry, and plant infectivity. Variability in the use of carbohydrates by isolates from a single plant species has been previously shown. Benson and Hanna (7) found unique utilization patterns for *Alnus incana* subsp. *rugosa* isolates which were divided into gel groups by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Furthermore, the utilization of the carbohydrates maltose, rhamnose, and trehalose by some of the isolates in this study suggests that these isolates are physiologically more versatile than other group B (see below) isolates previously described. An extensive study of *Frankia* isolates is required to understand more fully the relationship between *Frankia* subgroups which are determined by these various methods.

Frankia strains are presently assigned to the genus based on morphology and cell chemistry. Induction of nodule formation on host plants is useful, but the proposed definition of the species (17a) does not exclude noninfective strains. In this study, all the isolates examined were assigned to the genus *Frankia* based on morphological, chemical, and plant infectivity characteristics. The morphological criteria established for *Frankia* species (17) were met. The major differences observed among strains were variations in soluble pigment formation, vesicle expression, and mycelial compactness.

All the isolates had identical whole-cell sugar patterns of type D with xylose as a diagnostic sugar and contained *meso*-diaminopimelic acid, a diagnostic amino acid for *Frankia* (16), indicating a cell wall composition of type III. All the isolates were infective and effective on their host plant, although significant variations in acetylene reduction rates were found.

A description of the genus *Frankia* at the species level is currently not possible. Host plant specificity groups were proposed by Becking (5) prior to the isolation of pure cultures of *Frankia*. Since 1978, when the first strain was isolated and tested in plants, it has been found that the host specificity groups of Becking are no longer valid (17a). *Frankia* strains are divided into two major phenotypic groups, A and B, based on morphological, physiological, chemical, and plant infectivity data. This delineation has been confirmed by serology (4), DNA homology (1), and cross-inoculation studies. The strains evaluated in this study were characteristic of, and met criteria for, placement in group B. Such criteria include the ability to reinfect the original host plant and produce effective nodules, the production of colorless cells, the formation of vesicles in simple medium, and microaerophilic growth. Isolates from this group infect plants of the genera *Myrica*, *Comptonia*, and *Alnus*. That all the strains in this study belonged to a single group is not atypical of results from other studies of *Frankia* strains isolated from a single plant species, although both group A and B isolates have been isolated from a single host plant (18). The possibility that the isolation method itself is selecting for a particular class of *Frankia* cannot be discounted. In their discussion of *Frankia* physiological groups, Lechevalier and Lechevalier (17a) suggest that group A isolates are in fact endoparasites and not endosymbionts. The selective filtration method used in this study enriches for plant vesicle clusters from which *Frankia* species are isolated, and these clusters may preferentially harbor the true endosymbiontic and not endoparasitic forms of *Frankia* species.

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