FRANKIACEAE FAM. NOV. (ACTINOMYCETALES) WITH
ONE NEW COMBINATION AND SIX NEW SPECIES OF
THE GENUS FRANKIA BRUNCHORST 1886, 174

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ABSTRACT. Frankiaceae fam. nov. is proposed with the type genus Frankia. It includes in the genus Frankia one new combination, F. elaeagni and six new species, F. discariae, F. casuarinae, F. coriariae, F. dryadis, F. purshiae and F. cercocarpi.
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The purpose of this publication is to propose a new taxonomic treatment of an important group of bacteria, which produce nodules on the roots of non-leguminous dicotyledons. The root nodule, product of the symbiosis of host and bacteria, is able to fix atmospheric nitrogen. A single family, a single genus, and ten species are recognized.

Frankiaceae fam. nov.

Frankiaceae Modern L. n. Frankia, a generic name; -aceae M.L. pl. n. ending indicating name of a family; Frankiaceae the family name.

Obligate symbiotic organisms living in root nodules of a wide taxonomic range of non-leguminous dicotyledonous plants belonging to 6 orders, 7 families, and 14 genera (Table 1). Root nodules are produced by the symbiosis and these have the capacity to fix molecular nitrogen.

For the nitrogen-fixation process molybdenum is required; non-leguminous nodulated plants deficient in this element show restricted nitrogen fixation. Such plants grown under nitrogen-deficient conditions (in soil or water culture) produce only poor growth (Hewitt and Bond 1961, 159; Bond and Hewitt 1961, 1033; Becking

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TABLE 1. NON-LEGUMINOUS NODULE-BEARING PLANTS
WITH FRANKIA-SYMBIOSIS

Plant Order	Plant Family	Plant Genus	Frankia species
Fagales - - - -	Betulaceae - - -	<u>Alnus</u> - - - -	1. <u>alni</u>
Rhamnales - - - -	Elaeagnaceae - -	<u>Elaeagnus</u>	2. <u>elaegn</u>
		<u>Hippophaë</u>	
	Rhamnaceae - - -	<u>Shepherdia</u>	
		<u>Ceanothus</u> - - -	3. <u>ceanothi</u>
		<u>Discaria</u> - - -	4. <u>discariae</u>
Myricales - - - -	Myricaceae - - -	<u>Myrica</u>	5. <u>brunchorstii</u>
		<u>Gale</u>	
		<u>Comptonia</u>	
Casuarinales - -	Casuarinaceae -	<u>Casuarina</u> - - -	6. <u>casuarinae</u>
Coriariales - - -	Coriariaceae - -	<u>Coriaria</u> - - -	7. <u>coriariae</u>
Rosales - - - -	Rosaceae - - - -	<u>Dryas</u> - - - -	8. <u>dryadis</u>
		<u>Purshia</u> - - - -	9. <u>purshiae</u>
		<u>Cercocarpus</u> - -	10. <u>cercocarp</u>

1961a, 1204; 1961b, 217). Cobalt is also essential for nitrogen fixation (Bond and Hewitt 1962, 94; Hewitt and Bond 1966, 480). Vitamin B₁₂ analogues (containing cobalt) have been isolated from non-leguminous root nodules (Kliwer and Evans 1962, 108; Bond *cs* 1965, 319).

Optimal nitrogen fixation by the root nodules in some species at reduced oxygen pressure. In *Alnus* and *Hippophaë* at 12 per cent oxygen (Bond 1959, 59; 1961, 93), but in *Myrica* and *Casuarina* at about 20 per cent oxygen (Bond 1961, 93).

The taxonomic position of Frankiaceae has been uncertain. All species are filamentous and develop a mycelium. The family shows relationship both to the Actinomycetaceae and to the Streptomycetaceae, but is recognized as a distinct family on the basis of its morphological and physiological characteristics. The vegetative mycelium divides by segmentation into coccoid or polyhedral elements, a feature of the Actinomycetaceae. The mycelia also produce terminal swellings which may be regarded as degenerated conidia, perhaps corresponding to the conidia of the Streptomycetaceae. However, in contrast to these two families, cultivation in vitro on artificial media has been thus far unsuccessful. In an attempt to clarify relations to both families, there is under way a study of the major cell-wall components, which are used for the differentiation of aerobic actinomycetes (Lechevalier, Lechevalier and Becker, 1966, 151). This analysis may show its affinity to either the Actinomycetaceae or the Streptomycetaceae.

Growth in vitro on artificial media not yet obtained. All claims of isolations of the endophyte (von Plotho 1941, 1; Uemura 1952a, and 1952b, 209; 1961, 1; Pommer 1959, 138; Fiuczek 1959, 283; Wollum *cs* 1966, 463; Allen *cs* 1966, 57) have thus far not been confirmed by recent research. In most cases critical re-infection tests with the isolates were not carried out or gave negative results.

Growth in root-nodule tissue grown in vitro in tissue culture. Here the difficulty exists that the endophyte is not readily transmitted from the infected cells to newly formed callus tissue, so most explants lack the endophyte (Becking 1955, 885; 1966, 211).

Gram positive or Gram variable.

Probably micro-aerophilic.

Mesophilic.

TABLE 2. DIFFERENTIATION OF SPECIES OF FRANKIA

Species	Hyphae	terminal swelling of Hyphae		polyhedral-shaped cells	Studied by
		spherical	clubshaped		
1. <u>F. alni</u>	0.3 - 0.5 μ	3.0 - 5.0 μ rarely 6.0 - 8.0 μ	-	0.5 - 1.5 μ	EM, LM
2. <u>F. elaeagni</u>	0.3 - 0.5 μ	2.5 - 4.0 μ rarely 1.8 - 2.2 μ	-	0.3 - 0.9 μ	LM
3. <u>F. ceanothi</u>	0.3 - 0.4 μ	1.5 - 3.0 μ in average appr. 2.0 - 2.5 μ	in younger stages with slowly tapering base	unknown	EM [*] , LM
4. <u>F. discariae</u>	0.3 - 0.4 μ	appr. 4.0 μ	-	unknown	LM
5. <u>F. brunchorstii</u>	1.2 - 2.8 μ	-	length: 7.5 - 12.5 μ ; diameter at widest point: 1.6 - 2.4 μ	1.5 - 2.5 μ	EM, LM
6. <u>F. casuarinae</u>	0.3 - 0.5 μ	-	length: 3 - 4 μ ; diameter at widest point: 0.6 - 1.5 μ	0.4 - 1.0 μ	EM [*] , LM
7. <u>F. coriariae</u>	0.4 - 0.7 μ	-	length: 9 - 12 μ ; diameter at widest point: 1.2 - 1.3 μ	unknown	EM [*] , LM
8. <u>F. dryadis</u>	0.5 - 0.8 μ	-	length: 1.5 - 5.0 μ ; diameter at widest point: 1.5 - 2.0 μ	1.0 - 2.0 μ	EM [*] , LM
9. <u>F. purshiae</u>	0.3 - 0.5 μ	NOT YET STUDIED			LM
10. <u>F. cercocarpi</u>	0.3 - 0.5 μ	i d e m			LM

Note: EM = Electron microscopy

LM = Light microscopy

* = unpublished data of the author.

Inhabitants of plant tissue (symbiotically living), but also present in a free stage in soil. The latter stage must be a viable, probably a resting stage, since sterile non-legume seedlings nodulate when planted in suitable soil. The endophyte enters the plant through the root hair, which previously undergoes strong curvation by auxin production by the endophyte (Becking 1966, 211; 1968, 47). By means of an infection thread the endophyte reaches the cortex of the root and by proliferation of plant cells of a side-root primordium a root nodule is formed. Therefore, a root nodule is a deformed side root of inhibited or arrested growth. The infected cells are enlarged and have a large and misshapen nucleus which is probably polyploid (Becking 1966, 211; 1968, 47).

Genus Frankia Brunchorst 1886, 174

(Frankiella Maire and Tison 1909, 242)

Frank'.i.a M. L. f. n. Frankia generic name dedicated to A. B. Frank (1839-1900), Swiss microbiologist.

True mycelium is produced. The mycelium is septate, but branching is not necessarily correlated with cross-wall formation. Diameter of the hyphae in most species 0.3 - 0.5 μm , but in F. coriariae and F. dryadis thicker (up to 0.7 - 0.8 μm) and the coarsest in F. brunchorstii with 1.2 - 2.8 μm . In most cases the hyphae are too slender and lack refractivity in the host tissue for study by light microscopy. The electron micrographs show that the hyphae contain fine strands of DNA not enclosed by a nuclear membrane. They are procaryotic. Distinct membranous bodies most probably identical with the so-called plasmalemmasomes (Edwards and Stevens 1963, 414) or mesosomes (Fitz-James 1960, 507; Chen 1964, 125) of some streptomycetes and Gram positive bacteria can be observed.

The hyphae show very regularly terminal swellings spherical or club-shaped in form. These structures are absent in the center of the host cell, which is completely filled by the hyphal mass. They only occur near the periphery of the hyphal mass on the radially arranged hyphae close to the plant cell wall. The spherical bodies are often called "vesicles". Some authors regard the vesicles and the club-shaped structures as a kind of sporangium. They claim to have observed that vesicles break up

into smaller pieces, so-called "bacteroids" (Schaede 1933, 389), but there is no recent evidence for this view as vesicles lack complete cell walls and in the older stages they are disintegrated by the living host-cell cytoplasm surrounding them (Becking cs 1964, 343). At the base of the branches of the nodule there is a zone of infected cells, where the endophyte undergoes resorption by the host.

Mycelium sometimes fragments into small particles, 0.3 - 1.5 μ m, but in *F. brunchorstii* 1.5 - 2.5 μ m of irregular sizes and with angular faces producing polyhydrons. The particles have been termed "bacteroids" or "bacteria-like" cells. They never occur with vesicles or club-shaped structures in the same host cell. They always fill a host cell completely. They occur only in dead host cells or host cells which have not survived infection. By rupture of the host-cell wall these particles are released into adjacent host cells or into soil. They probably represent a resting stage, capable of surviving in soil and of propagating the endophyte.

The presence or frequency of polyhedral-shaped cells in the host tissue has some relation with facility to produce nodulation in water cultures of the host plants. Species like *Alnus*, *Hippophaë*, *Elaeagnus*, *Myrica* and *Casaurina* where such cells are common, easily nodulate when a suspension of crushed nodules is applied to their root system. But in species like *Ceanothus* or *Goriaria* where these cells have not yet been observed, nodulation occurs only when planted in habitat soil.

The vesicles or club-shaped structures are probably associated with nitrogen fixation as they are abundant in actively nitrogen-fixing tissues. These bodies are later assimilated and dissolved by the living tissue, leaving behind shrunken and distorted structures, so-called "ghosts", without contents (Becking cs 1964, 343). The main source of nitrogen must, however, be the living actively metabolizing vesicle, since the amount of nitrogen obtained from resorption of the vesicles by the host cytoplasm cannot account for the total nitrogen increase of the non-legume in a certain space of time.

The hyphae, vesicles or club-shaped structures are Gram variable. The polyhedral-shaped structures are Gram positive.

Host-plant specificity. The endophyte is specific for a certain plant genus or a group of related genera, such

as the Elaeagnaceae and Myricaceae. It is therefore appropriate to give these cross-inoculation groups or barriers of incompatibility the status of species. In contrast to the Rhizobiaceae associated with leguminous plants, these cross-inoculation groups are strict; so far no exceptions are known.

Root nodules: Two types of root nodules occur. In most plant genera by continuous branching of the deformed side root a coralloid nodule with reduced apical meristematic activity is produced. In Casuarina and representatives of the Myricaceae, however, the apex of each nodule lobe gives rise to a normal, but negatively geotropic root. The root nodules in these species become clothed with upward growing negatively geotropic rootlets. The rootlet is termed a rhizothamnion (Gr. shrub root).

The root nodules are perennial.

The type species is Frankia alni (Woronin) von Tubeuf.

1. Frankia alni (Woronin) von Tubeuf 1895, 118.

(Schinzia alni Woronin 1866, 6; Entorrhiza alni Weber 1884, 378; Plasmodiophora alni (Woronin) Möller 1885, 105; Frankia subtilis Brunchorst 1886, 174; Frankiella alni (Woronin) Maire and Tison 1909, 242; Aktinomyces alni Pekto 1910, 505; Actinomyces alni von Plötho 1941, 4; Nocardia alni Waksman 1941, 35; Proactinomyces alni Krassil'nikov 1949, 74 and 1959, 131; Streptomyces alni Fiuczek, 1959, 285).

al'.ni L.gen.fem.n. alnus the alder.

Obligate symbiont of root nodules of species of Alnus.

Hyphae, 0.3 - 0.5 μ m filamentous, branched and septate. Branching is not always correlated with cross-wall formation. Membranous bodies (mesosomes) observed by electron microscopy are very conspicuous and associated with cross-wall formation. Hyphae are embedded in a thick layer of polysaccharide (EM low electron dense) in the host cell and enclosed by a double membrane, the invaginated cytoplasmic membrane of the host cell.

Spherical vesicles, normally 3.0 - 5.0 μ m in diameter, but occasionally (e.g. in Alnus undulata) up to 6.0 - 8.0 μ m in diameter, as the terminus of hyphae at the periphery of the hyphal mass. Within the vesicles cell division proceeds uncoordinated and anomalously, so that mostly incomplete cell walls are formed. The number of septa increases with the growth of the vesicle;

mesosomes associated with these septa are numerous. In a later stage the vesicles become resorbed by the host, loosing their contents and leaving behind only their cell walls and membranes.

In contrast to this, the polyhedral-shaped cells are the result of complete cell division. The individual cells are, in general, arranged in groups, each of which is separated from its neighbours by a thick polysaccharide layer. The polyhedral cells are never absorbed by the host, because they occur only in dead host cells. In the initial stage they have thin cell walls, but in mature condition they are enclosed by a thick cell wall and there are lipid inclusions as reserve material. The polyhedral-shaped cells are released by rupture of the host-cell wall and probably function in the endophyte's survival and dispersal.

Habitat: In the cortical parenchymatous cells of root nodules of the host plants, but also in soil although its form in the latter is unknown.

Host plants: So far as investigated all Alnus species are nodulated. The genus Alnus itself, however, holds a remarkable position because it belongs to the Betulaceae with 6 other genera (Betulus, Corylus, Carpinus, etc.), none of which are nodule bearing. Well-known nodulated species are: Alnus glutinosa and A. incana (Europe), A. rubra (United States), A. jorullensis (South America), and A. japonica and A. sieboldiana (Japan). Incomplete compatibility resulting in poor nodulation and nitrogen fixation exists between the endophyte of A. glutinosa and A. rubra (Becking 1966, 211; 1968, 47) and between the endophyte of A. glutinosa and A. jorullensis (Rodriguez-Barrueco 1966, 103).

Root nodules of coralloid structure and up to 5-8 cm (or the size of a tennis ball) in diameter. In general, only present in the top layers of the soil.

Note: Of the endophytes of all non-legumes, that of Alnus is the best studied. Its morphology and its life cycle are therefore more completely described than for any other species.

The nitrogen-fixing capacity of these root nodules was definitely proved by water culture experiments and by ^{15}N -tests (Bond 1955, 303; 1959, 59; Becking 1966, 211; 1968, 47).

2. Frankia elaeagni (Schröter) comb. nov.

(Plasmodiophora elaeagni Schröter 1886, 134;

Frankiella elaeagni (Schröter) Maire and Tison (1909, 243; Tetramyxa elaeagni Yendo and Takase 1932, 114; Actinomyces elaeagni Roberg 1934, 482; Proactinomyces elaeagni (Roberg) Krassil'nikov 1949,

Obligate symbiont of root nodules of Elaeagnus, Hippophaë and Shepherdia species. A description of this species is not included as it has been described and named previously.

Habitat: In the cortical parenchymatous cells of root nodules of host plants, but also in soil although its form in the latter is unknown.

Host plants: All Elaeagnus, Hippophaë and Shepherdia species are nodulated. Well-known species are: Elaeagnus angustifolia, E. commutata, E. macrophylla, E. umbellata, Hippophaë rhamnoides, Shepherdia argentea, and S. canadensis. Complete compatibility exists between the endophyte and all hosts of these genera and species.

Root nodules of coralloid structure, up to 5 cm in diameter. Usually only present in the top layers of soil.

Note: The endophyte has not yet been studied by electron microscopy. The nitrogen-fixing capacity was proved by water culture experiments and ^{15}N -tests (Bond *cs* 1956, 501; Bond 1957b, 513; Gardner and Bond 1957, 305; Gardner 1958, 717; Bond 1958, 216).

3. Frankia ceanothi Atkinson 1892, 177.

Obligate symbiont of root nodules of species of Ceanothus.

A description of the morphology is not included as the species has been described and named previously.

Habitat: In the cortical parenchymatous cells of root nodules of the host plant, but also in soil although its form in the latter is unknown.

Host plants: Species of Ceanothus. This genus is confined to North America. Well-known nodulated species are: Ceanothus americanus, C. leucodermis, C. sanguineus, and C. velutinus. Nodulation is further reported for C. azureus, C. cordulatus, C. cuneatus, C. delilianus, C. divaricatus, C. diversifolius, C. fendleri, C. foliosus, C. greggii, C. griseus, C. impressus, C. incanus, C. integerrimus, C. jepsonii, C. microphyllus, C. oliganthus, C. ovatus, C. parvifolius, C. prostratus, C. rigidus, C. sorediatus, and C. thyrsiflorus. Of the about 50 described Ceanothus species, only about half have been investigated for nodulation.

Note: The nitrogen-fixing capacity of Ceanothus root nodules has been proved by pot experiments (Quick 1944, 827; Hellmers and Kelleher 1959, 275; Russell and Evans 1966, 164) and by ^{15}N -tests (Delwiche *cs* 1965, 1045; Bond 1967, 57).

4. Frankia discariae spec. nov.

Obligate symbiont of Discaria toumatou root nodules. Hyphae, 0.3 - 0.4 μm filamentous, branched and septate.

Spherical vesicles, approximately 4.0 μm in diameter (Morrison and Harris 1958, 1746).

Polyhedral-shaped cells unknown.

Habitat: In the cortical parenchymatous cells of root nodules of the host plant, but also in soil although its form in the latter is unknown.

Host plant: Discaria toumatou, endemic in New Zealand. Characteristic on loess, alluvial fans and gravel crees.

Root nodules of coralloid structure, up to 2-3 cm in diameter. Usually only present in top layers of soil.

Note: The endophyte has not yet been studied by electron microscopy. The nitrogen-fixing capacity of Discaria root nodules has been proved by ^{15}N -experiments (Morrison 1961, 945).

5. Frankia brunchorstii Möller 1890, 224.

(Aktinomyces myricae Peklo 1910, 505; Proactinomyces myricae (Peklo) Krassil'nikov 1949, 75, and 1959, 132). Obligate symbiont of root nodules of Gale, Myrica and Comptonia. The species has been described previously.

Habitat: In the cortical parenchymatous cells of root nodules of the host plants and in soil.

Host plants: Species of Gale, Myrica and Comptonia. Well known nodulated species are Gale palustris (= Myrica gale, Europe and Asia), Myrica cerifera (Southern United States), Myrica cordifolia (Southern Africa), Myrica javanica (Asia) (Becking, 1970), and Comptonia peregrina (= Myrica asplenifolia, Eastern United States).

There is incomplete compatibility between the endophyte of Gale palustris and Myrica cerifera and Myrica cordifolia (Gardner and Bond 1966, 161).

Rhizothamnia are produced, i.e. the meristem of each nodule lobe gives rise to a negatively geotropic root, so that the root nodule becomes clothed with upwards growing rootlets. It is likely (see Fig. 2a and 2b, Ziegler

1959/60, 28), that Comptonia peregrina has normal coralloid root nodules. The root nodules may attain sizes of 5-6 cm; they usually only occur in the uppermost layer of soil.

Note: The nitrogen-fixing capacity of these root nodules has been confirmed by pot experiments and ^{15}N -tests (Bond 1951, 447; 1959, 59; Leaf *et al.* 1959, 662 and Sloger and Silver 1965, 299).

6. Frankia casuarinae spec. nov.

Obligate symbiont of root nodules of species of Casuarina.

Hyphae 0.3 - 0.5 μm , filamentous, branched and septate.

Club-shaped endings of the hyphae, especially close to host-cell wall. The clubs have rounded ends and are about 3 - 4 μm in length with a diameter of 0.6 - 1.5 μm at the widest point.

Polyhedral-shaped cells, 0.4 - 1.0 μm

Habitat: In the cortical parenchymatous cells of root nodules of the host plants, but also in soil although its form in the latter is unknown.

Host plants: So far as investigated all Casuarina species in their native habitat are nodulated. Their distribution is tropical and subtropical. Well known nodulated species are Casuarina cunninghamiana, C. equisetifolia, C. glauca, C. junghuhniana, C. lepidophloia, C. montana, C. muricata, C. stricta, C. sumatrana, C. tenuissima, and C. triangularis. C. equisetifolia is a beach-growing species, extremely salt tolerant. Complete compatibility exists in reciprocal inoculations with the endophyte of all Casuarina species.

Rhizothamnia are formed, i.e. the meristem of each nodule lobe produces a negatively geotropic root, so that the root nodule becomes clothed with upward growing rootlets. The root nodules may attain sizes up to 6 - 7 cm or that of a tennis ball. They occur mainly in the top layers of the soil. The branching of the individual nodule lobes is not strictly dichotomous, 3 - 4 branches may arise from the same point.

Note: The nitrogen-fixing capacity was confirmed by water culture experiments and ^{15}N -tests (Bond 1957a, 373; 1958, 216; 1959, 59). Optimal nitrogen fixation was at 20 per cent oxygen, higher oxygen levels inhibited nitrogen fixation (Bond 1964, 600).

7. Frankia coriariae, spec. nov.

Obligate symbiont of root nodules of Coriaria species. Hyphae, 0.4 - 0.7 μm , filamentous, branched and septate.

Club-shaped extremities of the hyphae near the host-cell wall. The clubs have rounded ends and are about 9 - 12 μm in length with a diameter of 1.2 - 1.3 μm at the widest point.

Polyhedral-shaped cells unknown.

Habitat: In the cortical parenchymatous cells of root nodules of the host plants, but also in soil although its form in the latter is unknown.

Host plants: Coriaria myrtifolia in Southern Europe (Bond and Montserrat 1958, 474), C. japonica in Japan (Kataoka 1930, 209) and eight New Zealand species and their hybrids: Coriaria angustissima, C. arborea, C. kingiana, C. lurida, C. plumosa, C. pottsiana, C. pteridoides and C. sarmentosa (Burke 1963, 377).

Complete incompatibility exists between the endophyte of C. myrtifolia and C. japonica (Bond 1962, 1103), thus there appears to be a strong adaptation to particular host species.

Root nodules are of coralloid structure, up to 4-5 cm in diameter under natural conditions. They mostly occur in the top layers of soil. In Spain and in New Zealand the Coriaria species quickly occupy broken ground and disturbed areas (especially along roads).

Note: Nitrogen fixation by root nodules of Coriaria was proved by pot experiments (Kataoka 1930, 209), vermiculite cultures and ^{15}N -tests (Harris and Morrison 1958, 1812; Bond 1962, 1103).

8. Frankia dryadis Becking 1969, nov. spec.

Obligate symbiont of root nodules of Dryas.

Hyphae, 0.5 - 0.8 μm , filamentous, branched and septate.

Club-shaped extremities of the hyphae near the host-cell wall. The clubs have rounded ends and are approximately 1.5 - 5.0 μm in length with a diameter of 1.5 - 2.0 μm at the widest point. The smaller club-shaped structures are therefore more or less round and look like vesicles. Sometimes intermediates between hyphae and club-shaped structures are observed, apparently not full-grown stages of latter.

Polyhedral-shaped cells, 1.0 - 2.0 μm

Habitat: In the cortical parenchymatous cells of the host plants and in soil.

Host plants: Primary in Dryas drummondii in Alaska (Allen cs 1964, 54; Lawrence cs 1967, 793), but also in two other Dryas species at one locality (Glacier Bay, Alaska), i.e. in Dryas octopetala and D. integrifolia according to Sprague cited by Lawrence cs 1967, loc. cit. 800. However, in Europe (Scotland and on the continent) the common species Dryas octopetala is not nodulated.

Root nodules are coralloid, up to 2 - 3 cm in diameter. They are usually found 15 cm or more beneath the surface and often near the ends of tiny feeder rootlets, in general 30-50 cm away from the base of the stem. They were not found on recently germinated seedlings, but they began to appear in the 2nd and 3rd year on most plants. They never occur in silt soil, but only in sandy or rock soils or in gravel beds.

Note: The nitrogen-fixing capacity of Dryas root nodules was suspected from ecological studies (Crocker and Major 1955, 427; Crocker and Dickson 1957, 169; Schoenike 1957-58, 55), but it was proved by ^{15}N -tests (Lawrence cs 1967, 793).

9. Frankia purshiae spec. nov.

Obligate symbiont of root nodules of species of Purshia.

Hyphae, 0.3 - 0.5 μm , filamentous, branched and septate.

The presence of spherical vesicles or club-shaped structures and polyhedral-shaped cells has not yet been determined.

Habitat: In the cortical parenchymatous cells of root nodules of the host plants, but also in soil although its form in the latter is unknown.

Host plants: Purshia tridentata and P. glandulosa ranging along the West Coast of North America from Canada (Southern British Columbia) down to Mexico.

Root nodules of coralloid structure up to 3 - 4 cm in diameter. In general, more frequent in the top layers of soil.

Note: The nitrogen-fixing capacity of Purshia root nodules was suggested by pot experiments (Wagle and Vlamis 1961, 745). It was proved by ^{15}N -tests (Webster cs 1967, 392).

10. Frankia cercocarpi spec. nov.

Obligate symbiont of root nodules of Cercocarpus.

Hyphae, 0.3-0.5 μm , filamentous, branched and septate.

The presence of spherical vesicles or club-shaped structures and polyhedral-shaped cells has not yet been determined.

Habitat: In the cortical parenchymatous cells of the host plant and in soil.

Host plant: Cercocarpus betuloides from Western United States.

Root nodules are of coralloid structure up to 3-5 cm in diameter. Often equally distributed over the root system.

Note: The nitrogen-fixing capacity of Cercocarpus root nodules has been proved by pot experiments (Vlamis cs 1964, 73). ^{15}N -tests for nitrogen fixation have not yet been performed.

FURTHER COMMENTS

Maire and Tison (1909, 242) proposed Frankiella to replace Frankia (Brunchorst 1886, 174), because the generic name Frankia was regarded as illegitimate because used twice as generic name of Spermatophyta. The Moscow revision of the International Code of Nomenclature of Bacteria states that "new generic names in bacteriology should not be later homonyms of genera of algae, fungi, protozoa or viruses". Obviously the objection is not valid.

Certain generic names applied to the symbiont of the alder are those of genera of fungi, namely Schinzia (Woronin 1866), Entorrhiza (Weber 1884), and Plasmodiophora (Möller, 1885).

The species names Aktinomyces alni Peklo 1910, Actinomyces alni von Plotho 1941, Nocardia alni Waksman 1941, Proactinomyces alni Krassil'nikov 1949, and Streptomyces alni Fiuczek 1959 are synonyms of Frankia alni. In general, if the descriptions are related to laboratory strains, it can be disputed whether these species names are true synonyms, because it is rather likely that they do not represent the endophyte, but merely contaminants or misidentifications. This, because according to the present treatise, the true endophyte is an obligate symbiont not cultivable on artificial media.

For practical reasons, however, these names are still listed as synonyms. First, it is in some cases rather difficult to evaluate from the paper whether the given description is based on the isolate, or the endophyte inside the host cell, or on both. Secondly, if these names are placed on the list of nomina rejicienda, either as nomina confusa, nomina ambigua or nomina dubia, then according

to the Moscow revision of the Code (Provision 3, p. 482) a detailed statement of the case should be submitted to the Judicial Commission of the Nomenclature Committee for study and appropriate action. When such a name proposed for rejection has been provisionally rejected by the Judicial Commission, bacteriologists are authorized to reject it pending the decision of the next International Congress for Microbiology.

REFERENCES

- Allen, E.K., O.N. Allen, and L.J. Klebesadel. 1964.
An insight into symbiotic nitrogen-fixing plant associations in Alaska. Proc. Alaskan Sci. Conf., 14th, Anchorage, Alaska, 54-63.
- Allen, J.D., W.B. Silvester, and M. Kalin. 1966.
Streptomyces associated with root nodules of Coriaria in New Zealand. New Zealand J. Bot. 4:57-65.
- Atkinson, G.F. 1892. The Genus Frankia in the United States. Bull. Torrey Bot. Club 19, 171-177.
- Becking, J.H. 1961a. Molybdenum and symbiotic nitrogen fixation by alder (Alnus glutinosa Gaertn.). Nature (Lond n) 192, 1204-1205.
- _____. 1961b. A requirement of molybdenum for the symbiotic nitrogen fixation in alder (Alnus glutinosa Gaertn.). Plant and Soil 15, 217-227.
- _____. 1965. In vitro cultivation of alder root-nodule tissue containing the endophyte. Nature (London) 207, 885-887.
- _____. 1966. Interactions nutritionnelles plantes-actinomycetes. Rapport général. Ann. Inst. Pasteur (Paris) Suppl. 111, 211-246.
- _____. 1968. Nitrogen fixation by non-leguminous plants. Dutch Nitrog. Fert. Rev. No. 12, 47-74.
- _____. 1970. Plant-endophyte symbiosis in non-leguminous plants. Plant and Soil 32:611-654.
- _____, W.E. de Boer, and A.L. Houwink. 1964.
Electron microscopy of the endophyte of Alnus glutinosa. Antonie van Leeuwenhoek 30, 343-376.
- Bond, G., 1951. The fixation of nitrogen associated with the root nodules of Myrica gale L., with special reference to its pH relation and ecological significance. Ann. Bot. 15, 447-459.

- _____. 1955. An isotopic study of the fixation of nitrogen associated with nodulated plants of Alnus, Myrica, and Hippophaë. J. Exp. Bot. 6, 303-311.
- _____. 1957a. The development and significance of the root nodules of Casuarina. Ann. Bot. 21, 373-380.
- _____. 1957b. Isotopic studies of nitrogen fixation in non-legume root nodules. Ann. Bot. 21, 513-521.
- _____. 1958. Symbiotic nitrogen fixation by non-legumes. In: Nutrition of the legumes, ed. E.G. Hallsworth, Proc. Univ. Nottingham, 5th Easter School Agr. Sci., 359 pp., Butterworth Sci. Publ. London, 216-231.
- _____. 1959. Fixation of nitrogen in non-legume root-nodule plants. In: Utilization of nitrogen and its compounds by plants. ed. H.K. Porter, 13th Symp. Soc. Exp. Biol., 385 pp., Cambridge Univ. Press, London, 59-72.
- _____. 1961. The oxygen relation of nitrogen fixation in root nodules. Z. allg. Mikrobiol. 1, 93-99.
- _____. 1962. Fixation of nitrogen in Coriaria myrtifolia. Nature (London), 193, 1103-1104.
- _____. 1964. Isotopic investigations of nitrogen fixation in non-legume root nodules. Nature (London) 204, 600-601.
- _____. 1967. Nitrogen fixation in some non-legume root nodules. Phytol. (Buenos Aires) 24, 57-66.
- _____, J.F. Adams, and E. H. Kennedy. 1965. Vitamin B₁₂ analogues in non-legume root nodules. Nature (London) 207, 319-320.
- _____, and E. J. Hewitt. 1961. Molybdenum and the fixation of nitrogen in Myrica root nodules. Nature (London), 190, 1033-1034.
- _____, and E. J. Hewitt. 1962. Cobalt and the fixation of nitrogen by root nodules of Alnus and Casuarina. Nature (London) 195, 94-95.
- _____, J.T. MacConnell, and A.H. McCallum. 1956. The nitrogen-nutrition of Hippophaë rhamnoides L. Ann. Bot. 20, 501-512.
- _____, and P. Montserrat. 1958. Root nodules of Coriaria. Nature (London) 182, 474-475.
- Brunchorst, J. 1886. Über einige Wurzelanschwellungen, besonders diejenigen von Alnus und den Elaeagnaceen. Unters. Botan. Inst. Tübingen 2, 151-177.

- Burke, W.D. 1963. Note on the occurrence of nodules in the New Zealand species of Coriaria. New Zealand J. Bot. 1, 377-380.
- Chen, P.L. 1964. The membrane system of Streptomyces cinnamomensis. Amer. J. Bot. 51, 125-132.
- Crocker, R.L. and B.A. Dickson. 1957. Soil development on the recessional moraines of the Herbert and Mendenhall glaciers, South-Eastern Alaska. J. Ecol. 45, 169-185.
- _____, and J. Major. 1955. Soil development in relation to vegetation and surface age at Glacier Bay, Alaska. J. Ecol. 43, 427-448.
- Davenport, H.E. 1960. Haemoglobin in the root nodules of Casuarina cunninghamiana. Nature (London) 186, 653-654.
- Delwiche, C.C., P.J. Zinke, and C.M. Johnson. 1965. Nitrogen fixation by Ceanothus. Plant Physiol. (Lancaster) 40, 1045-1047.
- Edwards, M.R. and R.W. Stevens. 1963. Fine structure of Listeria monocytogenes. J. Bacteriol. 86, 414-428.
- Egle, K. and H. Munding. 1951. Über den Gehalt an Häminkörpern in den Wurzelknöllchen von Nicht-Leguminosen. Naturwissenschaften. 38, 548-549.
- Fitz-James, P.C., 1960. Participation of the cytoplasmic membrane in the growth and spore formation of Bacilli. J. Biophys. Biochem. Cytol. 8, 507-528.
- Fiuczek, M. 1959. Fixation of atmospheric nitrogen in pure cultures of Streptomyces alni (in Polish). Acta microbiol. Polon. 8, 283-287.
- Gardner, I.C. 1958. Nitrogen fixation in Elaeagnus root nodules. Nature (London) 181, 717-718.
- _____, and G. Bond. 1957. Observations on the root nodules of Shepherdia. Can. J. Bot. 35, 305-314.
- _____, and _____. 1966. Host plant-endophyte adaptation in Myrica. Naturwissenschaften 53, 161.
- Harris, G.P. and T.M. Morrison. 1958. Fixation of nitrogen-15 by excised nodules of Coriaria arborea Lindsay. Nature (London) 182, 1812.
- Hellmers, H. and J.M. Kelleher. 1959. Ceanothus leucodermis and soil nitrogen in Southern California Mountains. Forest Sci. 5, 275-278.
- Hewitt, E.J. and G. Bond. 1961. Molybdenum and the fixation of nitrogen in Casuarina and Alnus root nodules. Plant and Soil 14, 159-175.

- _____, and G. Bond. 1966. The cobalt requirement of non-legume root nodule plants. *J. Exp. Bot.* 17, 480-491.
- Kataoka, T. 1930. On the significance of the root-nodules of Coriaria japonica, A. Gr. in the nitrogen nutrition of the plant. *Jap. J. Bot.* 5, 209-218.
- Kliwer, M., and H.J. Evans. 1962. B₁₂ coenzyme content of the nodules from legumes, alder and of Rhizobium meliloti. *Nature (London)* 194, 108-109.
- Krassil'nikov, N.A. 1949. Guide to the Bacteria and Actinomycetes (in Russian). Akad. Nauk. SSSR. Moscow 830 pp.
- _____. 1959. Diagnostik der Bakterien und Actinomyceten. Gustav Fischer Verlag, Jena, 813 pp.
- Lawrence, D.B., R.E. Schoenike, A. Quispel, and G. Bond. 1967. The role of Dryas drummondii in vegetation development following ice recession at Glacier Bay, Alaska, with special reference to its nitrogen fixation by root nodules. *J. Ecol.* 55, 793-813.
- Leaf, G., I.C. Gardner, and G. Bond. 1959. Observations on the composition and metabolism of the nitrogen-fixing root nodules of Myrica. *Biochem. J.* 72, 662-667.
- Lechevalier, H., M.P. Lechevalier, and B. Becker. 1966. Comparison of the chemical composition of cell-walls of Nocardiae with that of other aerobic Actinomycetes. *Int. J. Syst. Bacteriol.* 16, 151-160.
- Maire, R., and A. Tison. 1909. La cytologie des Plasmodiophoracées et la classe des Phytomyxinae. *Ann. Mycol.* 7, 226-253.
- Möller, H. 1885. Plasmodiophora alni. *Ber. Deut. bot. Ges.* 3, 102-105.
- _____. 1890. Beitrag zur Kenntniss der Frankia subtilis Brunchorst. *Ber. Deut. bot. Ges.* 8, 215-224.
- Morrison, T.M. 1961. Fixation of nitrogen-15 by excised nodules of Discaria toumatou. *Nature (London)* 189, 945.
- _____, and G.P. Harris. 1958. Root nodules in Discaria toumatou Raoul Choix. *Nature (London)* 182, 1746-1747.
- Peklo, J. 1910. Die pflanzlichen Aktinomykosen. *Zentralbl. Bakteriol. Parasitenk. (Abt. II)* 27, 451-579.

- Plotho, O. von. 1941. Die Synthese der Knöllchen an den Wurzeln der Erle. Arch. Mikrobiol. 12, 1-18.
- Pommer, E.H. 1959. Über die Isolierung des Endophyten aus den Wurzelknöllchen von Alnus glutinosa Gaertn. und über erfolgreiche Re-Infektionsversuche. Ber. Deut. bot. Ges. 72, 138-150.
- Quick, C.R. 1944. Effects of snowbrush on the growth of Sierra gooseberry. J. Forestry 42, 827-832.
- Roberg, M. 1934. Über den Erreger der Wurzelknöllchen von Alnus und den Elaeagnaceen Elaeagnus und Hippophaë. Jahrb. wiss. Bot. 79, 472-492.
- Rodríguez-Barrueco, C. 1966. Fixation of nitrogen in root nodules of Alnus jorullensis H.B. and K. Phytol. (Buenos Aires) 23, 103-110.
- Russell, S.A. and H.J. Evans. 1966. The nitrogen-fixing capacity of Ceanothus velutinus. Forest Sci. 12, 164-169.
- Schaede, R. 1933. Über die Symbionten in den Knöllchen der Erle und des Sanddornes und die cytologischen Verhältnisse in ihnen. Planta 19, 389-416.
- Schoenike, R.E. 1957-58. Influence of mountain avens (Dryas drummondii) on growth of young cottonwoods (Populus trichocarpa) at Glacier Bay, Alaska. Proc. Minn. Acad. Sci. 25 and 26, 55-58.
- Schröter, J. 1885-1889. In F. Cohn's Kryptogamen-Flora von Schlesien. Bd. 3, Heft 3, Pilze, J.U. Kern's Verlag, Breslau, 814 pp.
- Sloger, C. and W.S. Silver. 1965. Note on nitrogen fixation by excised root nodules and nodular homogenates of Myrica cerifera L. In: Non-heme Iron Proteins: Role in Energy Conversion, ed. A. San Pietro, Symp. Spons. by Charles F. Kettering Res. Lab., 473 pp., Publ. Antioch Press, Yellow Springs, Ohio, U.S., 299-302.
- Tubeuf, K. von. 1895. Pflanzenkrankheiten durch Kryptogame Parasiten verursacht. Verlag J. Springer, Berlin, 599 pp., loc. cit. 117-118.
- Uemura, S. 1952a. Studies on the root nodules of alders (Alnus spp.). (IV). Experiment on the isolation of actinomycetes from alder nodules. Bull. Govt. Forest Exp. Sta. (Japan) 52, 1-18.
- _____. 1952b. Studies on the root nodules of alders (Alnus spp.). (V). Some new isolation methods of Streptomyces from alder nodules. Bull. Govt. Forest Exp. Sta. (Japan) 57, 209-226.

- _____. 1961. Studies on the Streptomyces isolated from alder root nodules (Alnus spp.). Sci. Rept. Agr. Forest Fisheries Res. Council (Japan) 7, 1-90.
- Vlams, J., A.M. Schultz, and H.H. Biswell. 1964. Nitrogen fixation by root nodules of western mountain mahogany. J. Range Management 17, 73-74.
- Wagle, R.F. and J. Vlams. 1961. Nutrient deficiencies in two bitterbrush soils. Ecology 42, 745-752.
- Waksman, S.A. 1961. The Actinomycetes, vol. 2, Classification, identification and descriptions of genera and species. Publ. Williams and Wilkins Co., Baltimore, U.S., 363 pp.
- Weber, C. 1884. Ueber den Pilz der Wurzelanschwellungen von Juncus bufonius. Bot. Ztg. 42, 369-379.
- Webster, S.R., C.T. Youngberg, and A.G. Wollum II. 1967. Fixation of nitrogen by bitterbrush (Purshia tridentata (Pursh) D.C.). Nature (London) 216, 392-393.
- Wollum, II, A.G., C.T. Youngberg, and C.M. Gilmour. 1966. Characterization of a Streptomyces sp. isolated from root nodules of Ceanothus velutinus Dougl. Proc. Soil Sci. Soc. Amer. 30, 463-467.
- Woronin, M. 1866. Über die bei der Schwarzerle (Alnus glutinosa) und der gewöhnlichen Garten-Lupine (Lupinus mutabilis) auftretenden Wurzelanschwellungen. Mém. Acad. Imp. Sci. St. Pétersbourg, Série 7, T. 10, No. 6, 1-13.
- Yendo, Y., and T. Takase. 1932. On the root nodule of Elaeagnus (in Japanese). Bull. Seric. silk Indust., Uyeda (Japan) 4, 114-134.
- Ziegler, H. 1959-60. Die Rhizothamnien bei Comptonia peregrina (L.) Coult. Mitt. Deut. Dendrol. Ges. 61, 28-31.