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Morphological Characterization of *Frankia purshiae*, the Endophyte in Root Nodules of Bitterbrush

Abstract

Histological analyses were made of root nodules of *Purshia tridentata* collected in Idaho and Utah. Hypertrophied parenchyma cells in the cortex of nodules contained an endophyte characterized by obovoid vesicles connected to ends of narrow hyphae. The taxon *Frankia purshiae* Becking (*Actinomycetales*) is recommended for this endophyte, at least until its life cycle is clarified.

Introduction

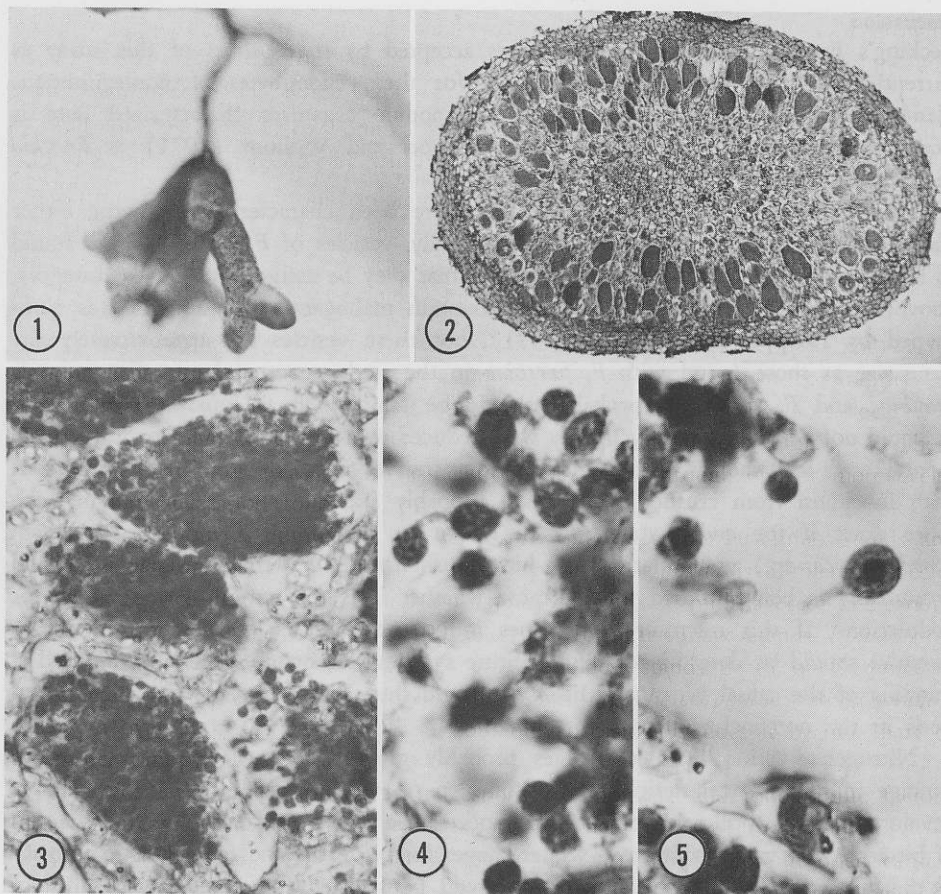
Antelope bitterbrush, *Purshia tridentata* (Pursh) DC., is one of the few rosaceous shrubs known to form root nodules that assimilate atmospheric nitrogen (Wagle and Vlamis, 1961; Webster *et al.*, 1967; and Becking, 1970). The micro-organism suspected of being mutualistically involved with bitterbrush in root nodules was recently classified by Becking (1970) as *Frankia purshiae* Becking, a member of a monogeneric family, Frankiaceae (*Actinomycetales*). Becking recognized ten species of *Frankia* and provided detailed descriptions of all except *F. purshiae* and *F. cercocarpi*. An endophyte in root nodules of mountain mahogany (*Cercocarpus* spp.) was recently described by Hoepfel and Wollum (1971). Presented here is a characterization of the endophyte in root nodules of *Purshia tridentata*.

Procedure

Root nodules of antelope bitterbrush were collected in northern Utah (K-1066, Cache Co., Logan Canyon, 13 September 1972) and central Idaho (K-1188, Lemhi Co., Cobalt, 4 October 1972, by B. Williams, J. D. Cass, and R. Brown). Fresh nodules were separated from the root systems, washed in tap water, cut into small branched segments, and immediately placed in formalin acetic acid (FAA). Later, nodule segments were dehydrated, embedded in paraffin, and sectioned at about 6 μ to 10 μ . Several stains proved useful, but best results were obtained with Peterson and Shurtleff's (1965) modified orseillin BB-aniline blue schedule. All measurements and photomicrographs were taken with light microscopy (Bausch & Lomb Model RCL-88 using 90x apochromat oil immersion objective) and the orseillin BB-aniline blue stain. A sample size of 50 was used for measurement of vesicles and hyphae.

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Figures 1-5. Figure 1. A simple coralloid root nodule on *Purshia tridentata* (X7). Figure 2. Nodule sectioned transversely just below a dichotomous branch. Dark, hypertrophied cells contain mycelia and vesicles of the endophyte along with host cell organelles (X45). Figure 3. Hypertrophied cells of the nodule cortex containing numerous vesicles (Longitudinal section, X500). Figure 4. Enlarged view of a portion of the preceding figure showing obovoid terminal vesicles on hyphae of the endophyte (X1600). Figure 5. A terminal vesicle isolated during sectioning. The dark central body within the vesicle resembles a nucleus, but the stain is not specific for nuclear materials (X1725).

Results

Root nodules were light tan to dark brown and generally coralloid. They varied from simple units ≈ 2 mm by 5 mm (Fig. 1) to complex coralloid aggregates ≤ 2 cm by 4 cm. Branching usually was dichotomous, and branches generally ranged from 0.5 mm to 1.0 mm in diameter by 3 mm to 6 mm in length.

Histological analyses of nodules revealed mycelial masses with terminal vesicles in hypertrophied parenchyma cells of the cortex (Figs. 2 and 3). Hyphae were 0.33μ to 0.55μ in diameter. Vesicle-filled cells were found as far as 2.6 mm from the nodule apex. Terminal vesicles were spherical to obovoid (Figs. 4 and 5). They were 2.2μ to 4.4μ wide and 2.2μ to 5.5μ long; average size of vesicles from the Utah collection was 3.3μ by 4.0μ and of those from the Idaho collection 3.0μ by 3.5μ . No polyhedral "bacterioides," common with several species of *Frankia*, were observed.