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Leaf nodule symbiosis: function and transmission of obligate bacterial endophytes

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Various plant species establish intimate symbioses with bacteria within their aerial organs. The bacteria are contained within nodules or glands often present in distinctive patterns on the leaves, and have been used as taxonomic marker since the early 20th century. These structures are present in very diverse taxa, including dicots (Rubiaceae and Primulaceae) and monocots (*Dioscorea*). The symbionts colonize the plants throughout their life cycles and contribute bioactive secondary metabolites to the association. In this review, we present recent progress in the understanding of these plant–bacteria symbioses, including the modes of transmission, distribution and roles of the symbionts.

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Introduction

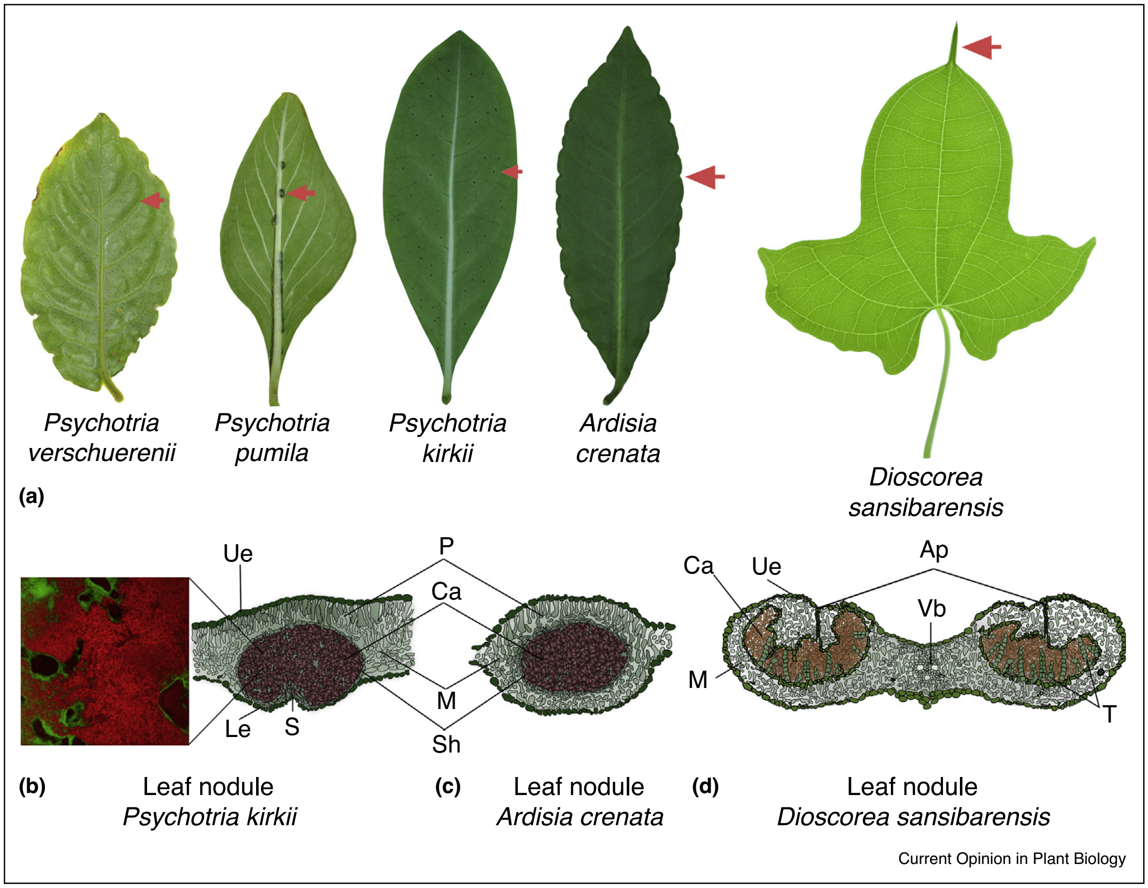
Leaf nodule symbiosis designates several symbiotic interactions affecting very diverse plant species with distinct geographical ranges [1]. The unifying characteristic of these symbioses is the presence of internal cavities in the leaf lamina harboring bacteria, often visible as nodules on the leaf surface [2]. Such structures have been reported in two dicot families, Rubiaceae (ca. 430 species) and Primulaceae (ca. 30 species) [3–6]. In addition, leaf glands are also found in at least one species of the monocot family Dioscoreaceae (Figure 1a) [7]. Even within a family, nodulated plants are polyphyletic and their geographical distribution does not necessarily overlap. For instance, nodulated members of the Rubiaceae family, which have been most intensively studied, fall in distinct genera also comprising non-nodulated members. Of these, the *Psychotria* genus contains 80 nodulated species, endemic to tropical and sub-tropical Africa and Madagascar [8,9], while the genus *Pavetta* contains ca. 350 nodulated species [10–12] distributed throughout Africa, Asia and New Zealand [11–13]. In the Primulaceae family, the presence of bacteria within nodules has been confirmed in 30 species of the *Ardisia* genus [11,12]. *Ardisia* species grow in a wide geographical range, but the nodulated species are limited to tropical Asia. *Dioscorea sansibarensis* (syn. *D. macroura*), a monocot, is also known to harbor leaf glands. It is native to Madagascar and tropical Africa, and invasive in Asia [7,14,15]. Although the anatomy of bacteria-containing nodules is distinct in this true yam species, the symbiosis has often been discussed together with leaf nodule symbioses of Rubiaceae and Primulaceae [7,11,12,15,16]. Leaf nodule symbioses represent some of the most intimate associations and may offer insights into the functions and rules which underlie the interaction between plants and bacteria in the phyllosphere.

We review here recent developments in the field of leaf nodule symbiosis, including the taxonomy and transmission of the bacterial symbionts as well as their potential ecological functions.

The symbiotic organ

The defining feature of leaf nodule symbiosis is the conspicuous organ containing the symbiotic bacteria. The shape, size and distribution of leaf nodules are species-specific and can vary widely depending on the species (Figure 1a–c) [3–6,13], but their ontogeny and general characteristics are analogous across plant genera. External environmental factors are not known to affect nodule characteristics, although heat may reduce the number of nodules in emerging leaves [4,12]. In Rubiaceae and Primulaceae species, symbiotic bacteria are contained in a mucilage layer in contact with specialized trichomes that surround the leaf primordia at the shoot tip. As new leaves emerge, bacteria colonize leaf tissue via stomatal openings and further divide in the sub-stomatal chamber to initiate nodule formation [5,17–19]. The leaves of aposymbiotic *Psychotria* and *Ardisia* species are devoid of nodules, which indicates that the presence

Figure 1



Morphology and distribution of leaf nodules. **(a)** Abaxial side of nodulated leaves of Rubiaceae, Primulaceae and Dioscoreaceae plant species. Nodules containing the symbiotic extracellular bacteria are indicated by red arrows. Schematic cross-section of mature leaf nodules in *P. kirkii* **(b)**, *A. crenata* **(c)** and *D. sansibarensis* **(d)**. A confocal laser scanning micrograph of a section of a *P. kirkii* nodule is shown in panel b. Plant cell walls are shown in green (stained with propidium iodide), bacteria in red (stained with acridine orange). Bacteria are embedded in a matrix or mucilage. In the schematic, plant cells are shown in green, bacteria in brown. Note the presence of mesophyll cells forming a network inside the *P. kirkii* and *A. crenata* nodules and the trichomes within the *D. sansibarensis* leaf gland providing a large contact area with bacteria. These are thought to enable the exchange of metabolites between the symbiotic partners. *Abbreviations*: Ue, upper epidermis; Le, lower epidermis; P, palisade cells; Ca, cavity filled with bacteria; M, mesophyll cells; Sh, cavity sheath; S, lower epidermal invagination; Ap, closed edges of the acumen aperture; Vb, main vascular bundle; T, secretory trichomes.
Source: Confocal microscopy image: Dr. Aurélien Bailly, University of Zurich.

of bacteria is necessary to initiate nodule formation [11,12].

In *D. sansibarensis*, nodule formation begins with a folding of the upper-epidermis at the tip of the leaf (Figure 1d), until bacteria become fully enclosed within channels containing numerous specialized trichomes. Symbionts are not in direct contact with the epidermis but are separated by a thick layer of epidermal cuticle wax [14,16]. Although there is little similarity in the structure of the leaf nodules, the shoot tips in nodulated dicot species also contain numerous modified secretory trichomes that arise from the lower epidermis, suggesting an important role of trichomes in host/symbiont interactions [16].

Identity of the symbiont and mode of transmission

Multiple attempts have been made to culture the bacterial symbionts of Rubiaceae and Primulaceae, without success [12,20–22]. Only recently have culture-independent molecular techniques provided a taxonomic affiliation to the *Burkholderia* genus, of the class β-proteobacteria. *Burkholderia* species are commonly isolated from the environment and also contain many host-associated members, including animal and human pathogens, but also β-rhizobia species capable of inducing nitrogen-fixing nodules on legume roots. The *Dioscorea* symbionts have been isolated and form the only species of the *Orrella* genus, a newly defined genus of the order *Burkholderiales* [15,20–24].