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EDITORIAL

Classic Spotlight: Bacteroids—Views of an Enigmatic Bacterial State in Root Nodule Symbiosis through the Centuries

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Rhizobia form an evolutionarily diverse group of plant symbiotic bacteria belonging to the *Alpha*- and *Betaproteobacteria*. These bacteria engage in symbiotic interaction with plants of the *Leguminosae* family and uniquely with the nonlegume genus *Parasponia* that culminates in bacterial nitrogen fixation to the benefit of the host (1). The root nodule symbiosis accounts for a large share of these interactions. In the course of this interaction, the newly induced plant organ is colonized by the bacteria, which penetrate the plant tissue through infection threads. These are plant-derived tubules filled with growing and dividing bacteria (2). During release from the infection threads, bacteria become enclosed in a plant-derived membrane and differentiate into so-called bacteroids. These are defined as endosymbiotic, morphologically distinct, nitrogen-fixing bacteria. Bacteroids provide combined nitrogen to the plant in exchange for nutrients. The first accurate morphological description of bacteroids dates back to Martinus Willem Beijerinck in his groundbreaking article describing root nodule bacteria of *Vicia* (3). He considered bacteroids to develop from bacteria that invade the roots, not as an autonomous formation of the protoplasm of the plant as believed by earlier researchers (3). Beijerinck suggested that during this metamorphic process bacteria gradually lose their ability to reproduce.

Debate over the viability and fate of bacteroids lasted over many decades. Early studies also provided divergent results regarding the DNA content of bacteroids (4–7). While some studies reported no differences in DNA content between free-living rhizobia and bacteroids, others reported an increase in DNA content in bacteroids. In a *Journal of Bacteriology* (JB) paper in 1977, Paau et al. (8) reported flow microfluorometry data unambiguously showing that bacteroids in the *Sinorhizobium meliloti*-*Medicago sativa* symbiosis have a higher DNA content than free-living cells. Diverse opinions on the fate and properties of bacteroids can be attributed mostly to the challenging experimental object and biological differences between specific rhizobium-legume interactions. After more than 125 years of further research, it is well known that root nodule symbiosis and bacteroid differentiation come in different flavors. Determinate or indeterminate nodule types are distinguished based on the transient or persistent character of host cell proliferation, respectively (9). Consequently, indeterminate nodules, continuously growing due to a persistent apical meristem, show a gradient of bacterial developmental stages along the nodule. In contrast, bacteria develop rather synchronously in determinate nodules. Terminally differentiated, enlarged, or even branched bacteroids are found mostly in indeterminate nodules, while bacteroids in determinate nodules remain small and are capable of resuming free-living growth (10). In both types of nodules, bacteroids show changes in gene expression and metabolic activity, while large changes in DNA content are found mainly during bacteroid differentiation in indeterminate nodules (10).

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Advanced electron microscopic analyses have promoted a significantly better understanding of early infection steps, release of bacteria from infection threads, and bacteroid differentiation. From the 1960s to the 1980s, several JB papers made major contributions in this field (11–15). These studies revealed the full spectrum of bacteroid morphologies and types of symbiosome, which is defined as one or more bacteroids enclosed in a peribacteroid membrane. Depending on the host plant, symbiosomes containing either a single bacteriod, e.g., in *M. sativa* nodules (11, 14), or multiple bacteroids, e.g., in *Glycine max* and *Vigna sinensis* nodules (12, 13), were observed. Paau et al. (15) performed a systematic study of the ultrastructure of *S. meliloti* cells in the different developmental zones of an indeterminate *M. sativa* nodule. From their fine-grained analysis, the authors concluded that vegetative bacterial cells released from the senescent nodule originate from the reservoir of bacteria in infection threads. They took the view that in spite of bacteroid degeneration, release of infection thread bacteria from senescent nodules increases the bacterial population in the soil, supporting a truly symbiotic relationship.

Discovery of antimicrobial nodule-specific cysteine-rich (NCR) peptides in the inverted repeat-lacking clade (IRLC) of legumes and their role in imposing irreversible terminal differentiation onto bacteria has recently opened an exciting new chapter in root nodule symbiosis research (10, 16, 17). This points to an evolutionary trend toward plant dominance over symbionts in this clade of legumes (18) and the importance of a reservoir of bacteria in infection threads capable of resuming growth after release from senescent indeterminate nodules. It is commonly speculated that this reservoir of infection thread bacteria is particularly important in light of terminal bacteroid differentiation in indeterminate nodules. In contrast, nodules with a determinate meristem contain very few persistent infection threads. If all bacteria in a determinate nodule were driven to terminal differentiation by NCR peptides, very few bacteria would survive nodule senescence. This would likely constitute a considerable selective disadvantage for the legume partner and may provide an explanation why the two types of nodules are different with respect to imposing terminal differentiation on bacteroids.

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