S47 Morphological characterization of *Sinorhizobium sp*. NGR234 bacteroids

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Symbiosis between legumes and rhizobia leads to the formation of root nodules where bacteria differentiate into intracellular nitrogen-fixing bacteroids. Indeterminate nodules (IDN) are elongated and have a persistent apical meristem, whereas mature nodules of determinate type (DN) are spherical and have no meristems. Depending on the host plant and the type of nodules that develop, variations exist in the nature on the differentiation state of the endosymbiotic microbes. For example, nodule-specific cysteine-rich (NCR) peptides of the galegoid legume *Medicago truncatula* were shown to push bacteroids of *Sinorhizobium meliloti* strain 1021 towards an irreversible differentiation by blocking bacterial cell division and triggering endoreduplication cycles (1,2). In contrast bacteroids of *R. leguminosarum* bv. *phaseoli* and *Mesorhizobium loti* that were isolated from DN of bean and *Lotus* respectively, were found to have morphological characteristics similar to free-living rhizobia and capable of forming colonies on plates (1,3).
Sinorhizobium sp. strain NGR234 is a promiscuous bacterium which forms symbiosis with a great variety of legumes. Therefore it is a good model to compare the effects of distinct nodule backgrounds on the morphology and physiological state of the nitrogen-fixing bacteroids. To this end, homogenous populations of NGR234 bacteroids were isolated via Percoll gradients from nodules of distinct hosts such as *Leucaena leucocephala* (IDN), *Tephrosia vogelii* (IDN) or *Vigna unguiculata* (DN). Isolated bacteroids were subsequently stained with fluorescent dyes and examined by Nomarski and fluorescence microscopy. 4′,6-diamidino-2-phenylindole (DAPI) was used to compare the size and shape of cells, whereas propidium iodide (PI) and the 5-cyano-2,3-di-4-tolyl tetrazolium chloride (CTC) redox dye were used to assess the integrity of membranes and monitor cell respiration, respectively. In addition, the DNA content of heat-killed cells stained with PI was measured with flow cytometry. Our data showed that bacteroids of NGR234 found in DN or IDN had active respiration and retained shapes and cell sizes similar to free-living cells. However, bacteroids isolated from IDN were (i) blocked in the G0/G1 phase of the cell cycle, (ii) almost incapable of forming colonies on agar plates, and had membranes with a modified permeability when compared to free-living bacteria. Thus, the fate of a promiscuous bacterium such as NGR234 differs considerably depending on the host. In addition, our results confirmed that legumes such as *T. vogelii* or *L. leucocephala*, which are not members of the galegoid tribe, also control bacteroid proliferation albeit yet in an unknown manner.

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