Biodiversity of uptake hydrogenase systems from legume endosymbiotic bacteria

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Abstract

Uptake hydrogenases in legume endosymbiotic bacteria recycle hydrogen produced during the nitrogen fixation process in legume nodules. Despite the described beneficial effect on plant productivity, the hydrogen oxidation capability is not widespread in the *Rhizobiaceae* family. Characterization of hydrogenase gene clusters in strains belonging to *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* reveals a similar overall genetic organization along with important differences in gene regulation. In addition, phylogenetic analysis of *hup* genes indicates distinct evolutionary origins for hydrogenase genes in Rhizobia.

Introduction

Root- and stem-nodule bacteria collectively called rhizobia are capable of fixing atmospheric nitrogen in association with leguminous plants. In this process, a large amount of hydrogen is released as an obligate by-product of the nitrogen reduction. This hydrogen production has been described as one of the major factors that affect the efficiency of symbiotic nitrogen fixation [1]. Certain Rhizobia induce in bacteroids (the nitrogen-fixing form of the bacterium) uptake hydrogenases that are able to partially or completely oxidize the hydrogen produced in nodules. This symbiotic hydrogen oxidation has been shown to reduce the energy losses associated with nitrogen fixation and to enhance productivity in certain legume hosts [2]. Although not completely elucidated, several mechanisms have been postulated to explain the beneficial effect associated with the symbiotic hydrogenase activity. Among them, hydrogenase reaction might protect nitrogenase from the detrimental effect of oxygen, prevent inhibition of the nitrogenase reaction by hydrogen or provide an additional source of energy in those strains where hydrogen oxidation is coupled with ATP generation [3].

From the above reasons, the hydrogen oxidation capability is considered as a desirable trait in Rhizobia strains. However, hydrogenase activity is not widely distributed in these bacteria. Hydrogenases are common among *Bradyrhizobium japonicum*, *Bradyrhizobium* sp. (*Lupinus*) and *Bradyrhizobium* sp. (*Vigna*) strains, which are the microsymbionts of soya beans, lupini and cowpeas respectively [4]. In *Azorhizobium caulinodans*, hydrogenase activity has been observed in *Sesbania rostrata* bacteroids as well as in free-living cultures under nitrogen fixation conditions, and hydrogenase genes have been detected in most *Azorhizobium* strains [5,6]. In the genus *Rhizobium*, hydrogen oxidation capability is widely represented among strains of *Rhizobium tropici*, mostly those belonging to subgroup IIb [7]. In contrast, just a few strains of *R. leguminosarum* bv. viciae display hydrogenase activity, whereas the Hup trait is apparently absent in *R. leguminosarum* bv. phaseoli and *R. leguminosarum* bv. trifolii [8]. Also, no hydrogenase positive strains have been consistently described within *Sinorhizobium* and *Mesorhizobium* genera [9]. In addition to the differences in the frequency of Hup^+ strains, each group displays different levels of hydrogenase activity. Whereas all *R. tropici* strains tested show very low levels of hydrogenase activity, irrespective of the legume host tested [10], values are very high in *A. caulinodans* and *B. japonicum* strains [6], which suggests the existence of differences among hydrogenase systems in these bacteria.

Diversity of hydrogenase gene clusters in Rhizobia

The six genera of Rhizobia belonging to the α-subclass of Proteobacteria are clustered in four deep branches represented by *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium-Sinorhizobium-Allorhizobium* as derived from 16 S rDNA sequence analysis [11]. A phylogenetic study of Hup^+ strains of *Azorhizobium*, *Bradyrhizobium* and *Rhizobium* showed the expected trees when 16 S rDNA sequences were aligned (Figure 1A). In contrast, phylogenetic trees obtained from hydrogenase structural genes *hupS* and *hupL* sequences revealed a strong diversity among *Bradyrhizobium* strains that did not parallel the phylogeny based on 16 S rDNA sequences [6] (Figure 1B). Whereas strains of *Bradyrhizobium* nodulating *Lupinus* sp. cluster with *B. japonicum hup* sequences, those nodulating *Vigna unguiculata* are found in a separated, deep branch. This distribution suggests distinct evolutionary origins for hydrogenase genes in *Bradyrhizobium*. In addition, the phylogenetic analysis of *hup* genes showed minimal distances between *hup*

Key words: *Azorhizobium*, *Bradyrhizobium*, *hup* gene, legume, nitrogenase, *Rhizobium*.

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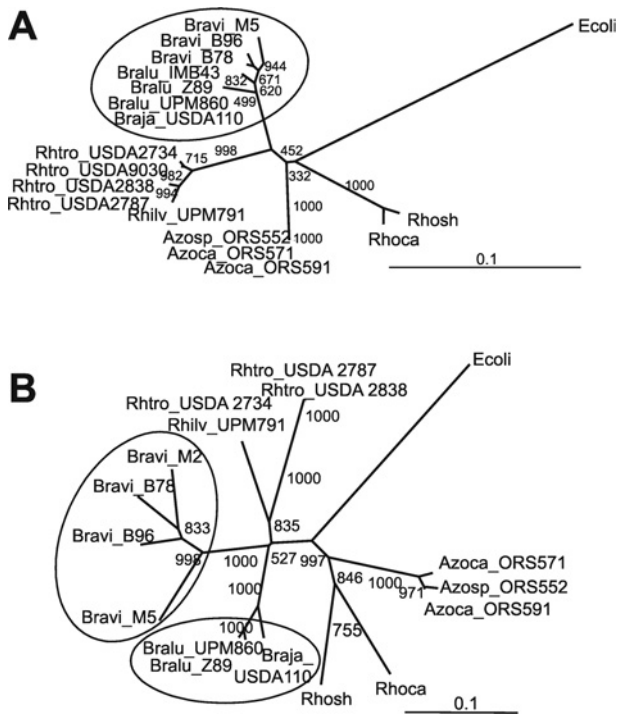
Figure 1 | Phylogenetic trees derived from 16 S rDNA and *hupS* sequences of Rhizobia

The minimum distance trees were generated by using CLUSTALX and TREEVIEW programs. Tree scales are indicated as per the site substitutions. Figures at nodes indicate bootstrap values (per 1000). Abbreviations: Rhilv, *R. leguminosarum* bv. viciae; Braja, *B. japonicum*; Bralu, *Bradyrhizobium* sp. (*Lupinus*); Bravi, *Bradyrhizobium* sp. (*Vigna*); Azoca, *A. caulinodans*; Azosp, *Azorhizobium* sp.; Rhtro, *R. tropici*; Ecoli, *Escherichia coli*; Rhoca, *R. capsulatus*; Rhosh, *R. sphaeroides*. Reproduced from [6] with permission. © (2002) American Society for Microbiology.



sequences from *R. tropici* and *R. leguminosarum*, lower than those observed for 16 S rDNA sequences.

Further analysis of *hup* gene composition in *Bradyrhizobium* sp. (*Vigna*), *Bradyrhizobium* sp. (*Lupinus*), *A. caulinodans*, *Azorhizobium* sp. and *R. tropici* strains by DNA hybridization revealed a high variability of hybridization patterns in *Bradyrhizobium*, whereas homogeneous intraspecific profiles were found for the *Azorhizobium* and *R. tropici* strains [6]. In this latter organism, the hydrogenase gene cluster is consistently located in the symbiotic plasmid [6], a situation similar to that found for *R. leguminosarum* Hup$^+$ strains [12]. This unusual degree of *hup* gene conservation could indicate that *hup* genes have been recently acquired in *Rhizobium*. Hybridization assays with *hup* gene probes also highlighted a different composition in regulatory genes among these genera [6]. The *hupUV* genes encode the regulatory hydrogenase that controls expression of *hupSL* genes in *B. japonicum* [13]. These genes were detected in *Bradyrhizobium* sp. (*Lupinus*), *Azorhizobium* sp. and *A. caulinodans* strains, but not in *Bradyrhizobium* sp. (*Vigna)* or *R. tropici* [6].

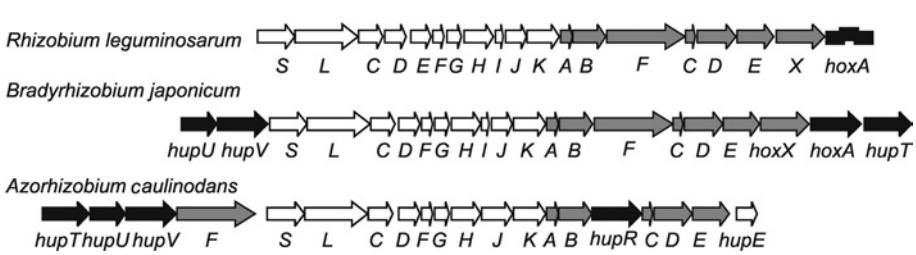
A comparative analysis of hydrogenase systems from *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*

A detailed molecular characterization of hydrogenase gene clusters has been performed in *R. leguminosarum*, *B. japonicum* and, recently, in *A. caulinodans* [14,15]. In all cases *hup* genes are contained in large DNA regions of approx. 20 kb with a similar genetic composition (Figure 2). Downstream of the structural genes, a set of *hup* and *hyp* genes coding for proteins with accessory functions is found. The arrangement of these genes is basically the same in *R. leguminosarum* and *B. japonicum* [14]. In contrast, the genetic organization in *A. caulinodans* is slightly different and resembles that of *hup* genes in the phylogenetically close bacterium, *Rhodobacter capsulatus* [15]. Both *hup* gene clusters share three features: *hypF* is found upstream of the structural genes, *hypX* is absent and *hupIhupJ* genes are fused encoding a single protein. Characteristic of the *A. caulinodans hup* cluster is the presence of the *hupE* gene, which has been identified in few hydrogenase systems, including that of *R. leguminosarum* [14,15].

As previously mentioned for hybridization assays with *hup* gene probes, major differences in these genera are related to the presence or absence of specific hydrogenase regulatory genes. Although *B. japonicum* and *A. caulinodans* possess a regulation system formed by the *hupUhupVhoxA(hupR)* and *hupT* gene products [13–15], such a system has not been identified in *R. leguminosarum* [14]. Moreover, a truncated non-functional copy of the *hoxA* gene was found in this

Figure 2 | Genetic organization of hydrogenase clusters in *R. leguminosarum* bv. viciae UPM791, *B. japonicum* USDA110 and *A. caulinodans* ORS571

White, grey and black arrows indicate *hup*, *hyp* and regulatory genes respectively.



organism, suggesting that *hup* gene regulation has evolved to be exerted by the master activator of the nitrogen fixation process, NifA [14]. The occurrence of different regulatory pathways for hydrogenase expression in Rhizobia correlates with the ability of *B. japonicum* and *A. caulinodans* to induce hydrogenase activity in free-living cultures and the lack of expression in *R. leguminosarum* vegetative cells. Further molecular characterization of newly described *hup* gene clusters in *Bradyrhizobium* sp. and *R. tropici*, currently in progress, will shed light on the evolution of hydrogenases in Rhizobia.

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