

Diversity of nickel ligands in nodule cytosol, nickel transport, and expression of a nickel-dependent enzyme in endosymbiotic bacteria as affected by the legume host

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Summary

Provision of metals to endosymbiotic bacteria represents a potential limitation for metalloenzyme synthesis inside legume nodules. Metal ions are usually bound to organic ligands in the cell cytoplasm, and the nature of such metal-ligand complexes might affect metal availability. We have observed a strong effect of the legume host on hydrogenase synthesis when the same *Rhizobium leguminosarum* bv. *viciae* strain establishes a symbiotic interaction with pea (*Pisum sativum*) or lentil (*Lens sculenta*) plants. These data, along with the different phenotypes of mutants altered in nickel (Ni) transport in these hosts, suggest a role for the chemical form of Ni on metal provision to the bacteroid. The biochemical analysis of cytosolic fractions of pea and lentil nodules has revealed the different nature and concentration of organic ligands chelating Ni in these hosts.

Introduction

In natural environments metals are often chelated by organic ligands due to their high binding affinity (Waldron & Robinson, 2009). In the case of endosymbiotic bacteria, bacteroids are surrounded by plant cytoplasm in which metal ions are also complexed by different ligands. Some of these ligands are organic acids, which, at the same time, are the main carbon substrate transferred to bacteroids in legume nodules (den Herder & Parniske, 2009). We are interested in the effect of such complexes on the supply of metals to the bacteroids. Previous reports indicated that the synthesis of [NiFe] hydrogenase in the nodules is limited by the availability of Ni (Brito *et al.*, 1994; Ureta *et al.*, 2005). Also, expression of this metalloenzyme is strongly affected by the legume host (López *et al.*, 1983; Brito *et al.*, 2008). In this work we have studied the nature of organic acids complexing Ni in the cytosol fraction of nodules induced in different legume hosts, correlating it with Ni transport and hydrogenase activity in bacteroids.

Materials and Methods

The *R. leguminosarum* wild type SPF25(pALPF1) and *hupE*-deficient mutant SPF22A(pALPFE) strains were previously described (Brito *et al.*, 2010). Organic ligands present in nodule cytoplasm were resolved by HPLC-UV/MS using a C18 reversed phase column. For separation and identification of Ni complexes, a silica column coupled to UV/MS was used. Identification of Ni compounds in the chromatography profile was performed by ICP-MS (Cacho *et al.*, 2010). Hydrogenase activities from bacteroids were determined by an amperometric method using oxygen as electron acceptor.

Results and Discussion

Two lines of evidence indicate that host-dependent differences in the chemical form of Ni in the nodule cytoplasm affects the expression of NiFe hydrogenase. First, we found that the lack of expression of hydrogenase in a non-permissive legume host can be partially complemented by the addition of Ni to the plant nutrient solution. Second, we observed differences in hydrogenase phenotype from a *R. leguminosarum* mutant deficient in the Ni transporter HupE when in symbiosis with pea vs. lentils, suggesting a different pathway of Ni uptake potentially due to the presence of different Ni ligands in both plants (Figure).

We have determined the nature of Ni complexes present in the cytosol of pea and lentil nodules induced by the same *R. leguminosarum* bv. *viciae* strain. By HPLC-UV/MS using a C18 reversed phase column we have observed that cytosol fractions of pea and lentil nodules differs in organic acid composition; however, Ni complexes could not be resolved by this approach. A new methodology using a normal phase chromatography with a silica column and a hexane:ethanol mobile phase coupled to UV/MS has been developed. By this method we have determined that Ni-malate and Ni-citrate are the main Ni complexes in pea nodules. In contrast, in nodules induced by the same strain in lentil plants, Ni is present mostly as Ni-citrate, although low amounts of Ni-malate and Ni-tartrate are also present. These results are consistent with the concentrations of each free ligand in the nodule cytosol of these hosts as corrected by the affinity constant of each ligand for Ni.

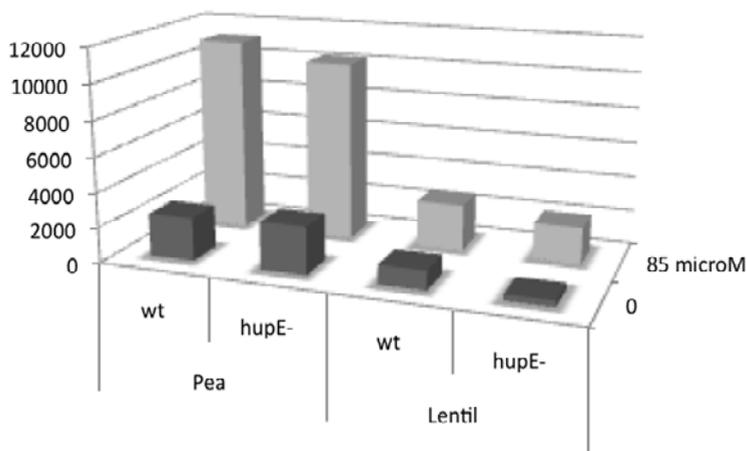


Figure. Effect of host and Ni transport on symbiotic hydrogenase activity. The graph shows the values of hydrogenase activity of pea and lentil bacteroids induced by *R. leguminosarum* SPF25(pALPF1) wild-type strain or by its *hupE*-deficient derivative SPF22A(pALPFE). Pea (*Pisum sativum* cv Frisson) and lentil (*Lens sculenta* cv Magda) plants were grown in nutrient solutions without Ni supplementation or supplemented with 85 μ M NiCl₂. Values are expressed in nmoles of H₂ oxidized per hour per mg protein, and represent the average of at least two experiments (adapted from Brito *et al.*, 2010).

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References

- Waldron KJ, Robinson NJ (2009) *Nat Rev Microbiol* 7: 25-35.
- Brito B, Toffanin A, Prieto RI, Imperial J, Ruiz-Argüeso T, Palacios JM (2008) *Mol Plant-Microbe Interact* 21: 597-604.
- Brito, B., R. I. Prieto, E. Cabrera, M. A. Mandrand-Berthelot, J. Imperial, T. Ruiz-Argüeso, Palacios JM (2010) *J Bacteriol* 192: 925-935.
- Brito B, Palacios JM, Hidalgo E, Imperial J, Ruiz-Argüeso T (1994) *J Bacteriol* 176: 5297-5303.
- Cacho C, Brito B, Palacios JM, Cámara C, Pérez-Conde C (submitted to *Talanta*).
- den Herder G and Parniske M (2009) *Curr Op Plant Biol* 12: 491-499.
- López M, Carbonero V, Cabrera E, Ruiz-Argüeso T (1983) *Plant Sci Lett* 29: 191-199.
- Ureta A, Imperial J, Ruiz-Argüeso T, Palacios JM (2005). *Appl Environ Microbiol* 71: 7603-7606.