# DmeRF system is required for nickel and cobalt resistance in *Rhizobium leguminosarum* bv. *viciae*.

Rubio-Sanz, L.[1*], Prieto, R.I.[2], Palacios, J.M.[2], Brito, B.[2]
[1] Departamento de Química y Análisis Agrícola, E.U. Ingeniería Técnica Agrícola, [2] Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Ciudad Universitaria s/n, 28040 Madrid. [1, 2] CBGP, Campus de Montegancedo, Carretera M40-Km 37.7, Pozuelo de Alarcón, 28223 Madrid, Spain.
[*] laura.rubio@upm.es

## ABSTRACT
A member of the Cation Diffusion Facilitator (CDF) family with high sequence similarity to DmeF (Divalent metal efflux) from *Cupridavirus metallidurans* was identified in *Rhizobium leguminosarum* bv. viciae UPM1137. The *R. leguminosarum dmeF* mutant strain was highly sensitive to $Co^{2+}$ and moderately sensitive to $Ni^{2+}$, but its tolerance to other metals such as $Zn^{2+}$, $Cu^{2+}$ or $Mn^{2+}$ was unaffected. An open reading frame located upstream of *R. leguminosarum dmeF*, designated *dmeR*, encodes a protein homologous to the nickel and cobalt regulator RcnR from *E.coli*. Expression of the *dmeRF* operon was induced by nickel and cobalt ions in free-living cells, likely by alleviating DmeR-mediated transcriptional repression of the operon.

## INTRODUCTION
Nickel and cobalt are essential microelements that participate in a variety of cellular processes. These two elements are usually found at low concentrations in soils. However, these metals can become toxic at moderate concentrations (Macomber *et al*., 2011). Metal homeostasis requires the balance of import and export pathways to control metal concentration inside the bacterial cell. Active transport through efflux systems represents the largest category of metal resistance systems (Bruins *et al*., 2000). Cation Diffusion Facilitators (CDF) constitutes one of the most important groups of efflux systems involved in this process.

## MATERIAL AND METHODS
*Estimation of metal resistance by disk diffusion tests*. The zone of inhibition of TY plate cultures around disks soaked in 200 mM $NiCl_2$, 100 mM $CoCl_2$, 500 mM $ZnSO_4$ and $CuSO_4$, and 100 µM $MnCl_2$ was determined as described (Bauer *et al*., 1966).

## RESULTS AND DISCUSSION
*Identification of a genetic system involved in nickel and cobalt resistance.*
A random mutagenesis of the metal-resistant *R.leguminosarum* bv. viciae strain UPM1137 allowed the identification of an ORF showing significant sequence identity to DmeF from *Cupridavirus metallidurans. Rhizobium leguminosarum* bv viciae *dmeF* encodes a 323 amino acid protein whose structural analysis using HMMTop predicts the existence of six transmembrane domains (TMD), two motifs characteristic of CDF proteins, and a histidine-rich stretch located between TMD4 and 5 (Figure 1). The ORF located upstream of DmeF encoded a protein with a 39% sequence identity to the nickel and cobalt regulator RcnR from *E. coli*, one of the founding members of the RcnR/CsoR structural class of transcriptional regulators (Iwig *et al*., 2006). Alignment of both proteins revealed that the *R. leguminosarum* gene product (designated DmeR) contained conserved cysteine and histidine residues involved in response to $Ni^{2+}$ and $Co^{2+}$ (Higgings *et al*., 2013).

*Phenotypic analysis of R. leguminosarum dmeRF mutant strains.*

Inactivation of *dmeRF* system in *R. leguminosarum* strains UPM1137 and SPF25 resulted in significant reduction of tolerance to nickel and cobalt ions, but not to other metals ($Mn^{2+}$, $Cu^{2+}$, $Zn^{2+}$). The mutation had no significant effects on symbiotic performance when plants were grown with standard nutrient solutions. In the case of plants supplemented with cobalt, however, mutation of *dmeRF* system resulted in a statistically significant decrease of the average values of shoot dry weight.

Expression analysis using *lacZ* fusions and qRT-PCR determinations revealed that expression of *dmeRF* operon was induced in response to the presence of Ni and Co, likely by alleviation of DmeR-dependent transcriptional repression.

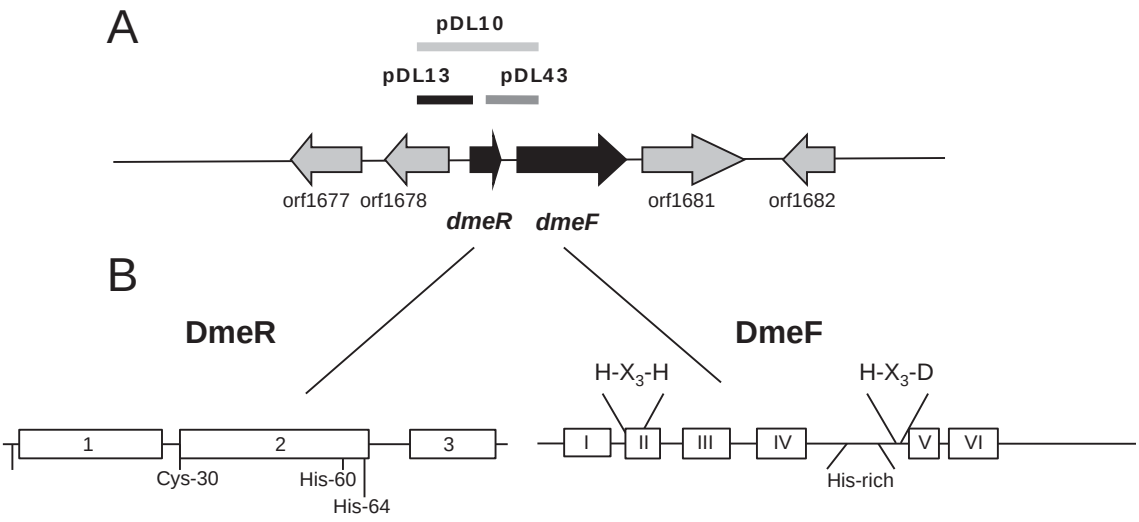


**Figure 1.** Analysis of *R. leguminosarum dmeRF* genes. A) Genomic context of *dmeRF* genes in the chromosome of *R.leguminosarum* UPM791. Lines above the genetic map indicate the DNA regions cloned in pMP220 in the indicated fusion constructs. B) Structural prediction of gene products as derived from bioinformatic analysis of *dmeR* and *dmeF* gene products are presented below the genetic map. Open bars indicate DmeR alpha helices as predicted by i-Tasser, and DmeR transmembrane domains as predicted by HMMTop. Relevant residues or motifs are indicated.

## ACKNOWLEGMENTS

This work was supported by Spain's MICINN BIO2010-15301 (J.M.P) and Proyecto de Creación y Consolidación de Grupos CCG10-UPM/GEN-5493 from Comunidad Autónoma de Madrid and Universidad Politécnica de Madrid (B.B.)

## REFERENCES

Bauer, A.W., *et al.* (1966). Am. J. Clin. Pathol. 45: 493-496.
Bruins, M.R., *et al.* (2000). Ecotoxicol. Environ. Saf. 45: 198-207.
Higgins, K.A., *et al.* (2013). Biochemistry. 52: 84-97.
Iwig, J.S., *et al.* (2006). Mol. Microbiol. 62: 252-262.
Macomber, L., and Hausinger, R.P. (2011). Metallomics 3: 1153-1162.