



Riboregulation of metabolism in nitrogen-fixing symbiotic α -rhizobia

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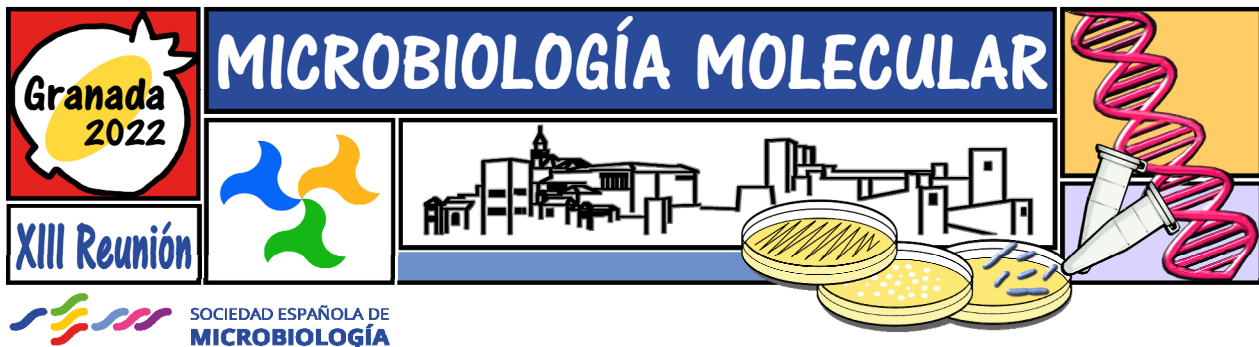
Nitrogen-fixing root nodule symbioses between rhizobia and legume plants provide more than half of the combined nitrogen incorporated annually into terrestrial ecosystems, rendering plant growth independent of environmentally unfriendly chemical fertilizers. Success of symbiosis primarily depends on the capacity of rhizobia to establish competitive populations in soil and rhizosphere. To date, genetic and metabolic reprogramming of rhizobia from free-living bacteria to nitrogen-fixing bacteroids onto nodules have been studied almost exclusively from the perspective of the transcriptional control orchestrated by proteins. However, post-transcriptional regulation of gene expression by small RNAs (sRNAs) is expected to play major roles in the establishment of these mutualistic symbioses¹.

Here, we deciphered a posttranscriptional network regulated by the homologous *trans*-sRNAs AbcR1 and AbcR2, which rewires metabolism of the nitrogen-fixing α -rhizobium *Sinorhizobium meliloti* during pre-infection stages of symbiosis with its legume host alfalfa². MS2-affinity purification coupled with RNA sequencing unveiled exceptionally large and overlapping AbcR1/2 mRNA interactomes, jointly representing ~6% of the *S. meliloti* protein-coding genes. Most mRNAs encode transport/metabolic proteins whose translation is silenced by modifiable base-pairing to two distinct anti-Shine Dalgarno motifs that function independently in both sRNAs. A metabolic model-aided analysis of the targetomes predicted changes in AbcR1/2 expression driven by shifts in carbon/nitrogen sources, which were confirmed experimentally.

AbcR1/2 interactomes are well represented in rhizosphere-related *S. meliloti* transcriptomic signatures. Remarkably, lack of AbcR1 specifically compromised the ability of *S. meliloti* to colonize the root rhizoplane. The AbcR1 regulon likely ranks utilization of available substrates to optimize metabolism, thus conferring *S. meliloti* an advantage for efficient rhizosphere/rhizoplane colonization. Because RNA regulation relies on modifiable base-pairing interactions, our findings open unexplored avenues for engineering the legumes rhizobiome within sustainable agricultural practices.

1. Robledo M, García-Tomsig NI, Jiménez-Zurdo JI (2020) Riboregulation in Nitrogen-Fixing Endosymbiotic Bacteria. *Microorganisms* 10;8(3):384.
2. García-Tomsig NI, Robledo M, diCenzo GC, Mengoni A, Millán V, Peregrina A, Uceta A, Jiménez-Zurdo JI (2022) Pervasive RNA Regulation of Metabolism Enhances the Root Colonization Ability of Nitrogen-Fixing Symbiotic α -Rhizobia. *mBio* 15;13(1):e0357621.

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