

# Non-Ionic Osmotic Stress Induces the Biosynthesis of Nodulation Factors and Affects Other Symbiotic Traits in *Sinorhizobium fredii* HH103

Francisco Fuentes-Romero<sup>1</sup>, Isamar Moyano-Bravo<sup>1</sup>, Paula Ayala-García<sup>1</sup>, Miguel Ángel Rodríguez-Carvajal<sup>2</sup>, Francisco Pérez-Montaña<sup>1</sup>, Sebastián Acosta-Jurado<sup>3</sup>, Francisco Javier Ollero<sup>1</sup>, José María Vinardell<sup>1,\*</sup>

## List of Supplementary Material

**Figure S1.**  $\beta$ -galactosidase activity of *S. fredii* HH103 carrying plasmid pMP240 upon growth in YM supplemented with different mannitol concentration (from 55 mM to 1 M).

**Figure S2.** Growth curves of *S. fredii* HH103 in different media supplemented or not with 400 mM mannitol. (A) TY medium; (B) YM medium; (C) Minimal medium (MM); (D) Bromfield medium (BF).

**Figure S3.** LPS (A) and KPS (B) electrophoretic profiles of *S. fredii* HH103 grown in TY medium in the absence or presence of 400 mM mannitol.

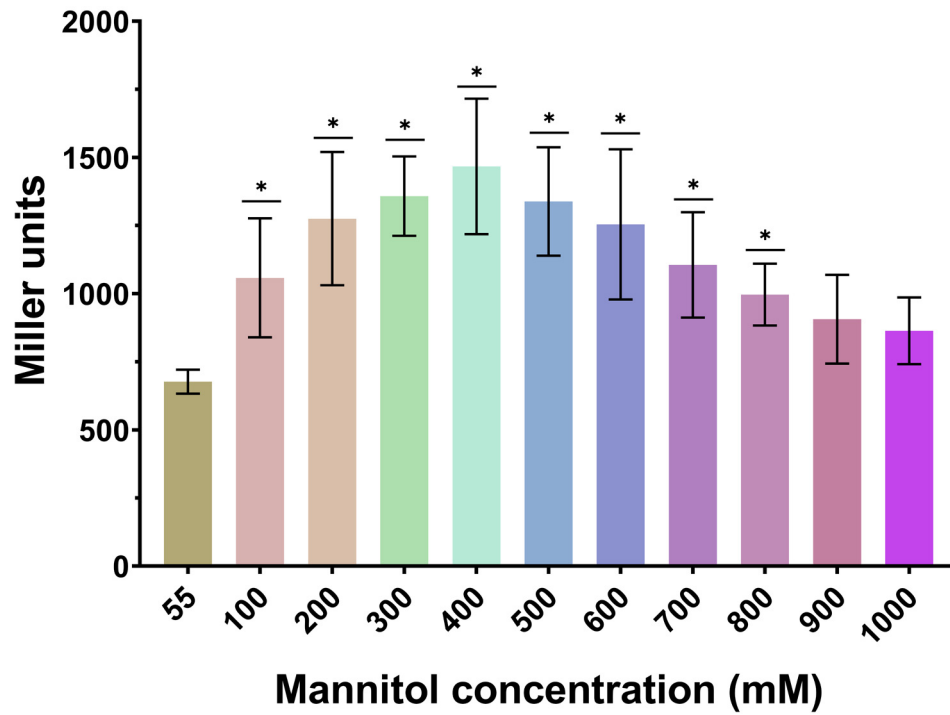
**Table S1.** Fold-changes, as determined by RNAseq, of the *S. fredii* HH103 *nodABC* genes after 16-h growth in the presence of 400 mM mannitol.

**Supplementary Dataset S1.** Complete list of Nod factors produced by *Sinorhizobium fredii* HH103 in YM medium in the absence (HH-) or the presence of 3.7  $\mu$ M genistein (HH+) or 400 mM mannitol (HH mannitol).

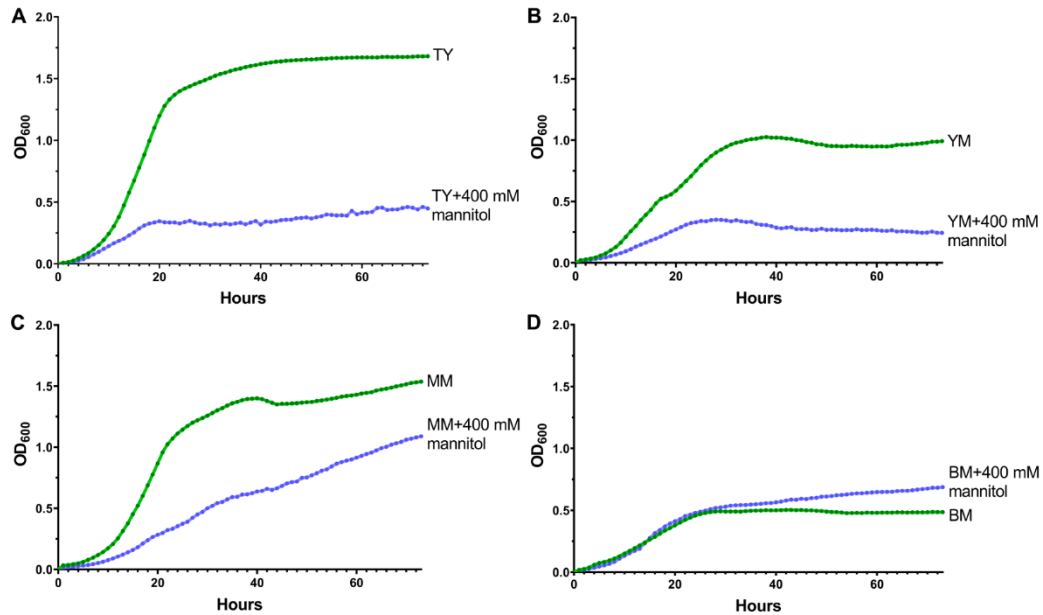
**Supplementary Dataset S2.** *S. fredii* HH103 DEGs upon growth in 400 mM mannitol ordered by fold-change.

**Supplementary Dataset S3.** List of *S. fredii* HH103 induced genes in the presence of 400 mM mannitol, ordered by replicon.

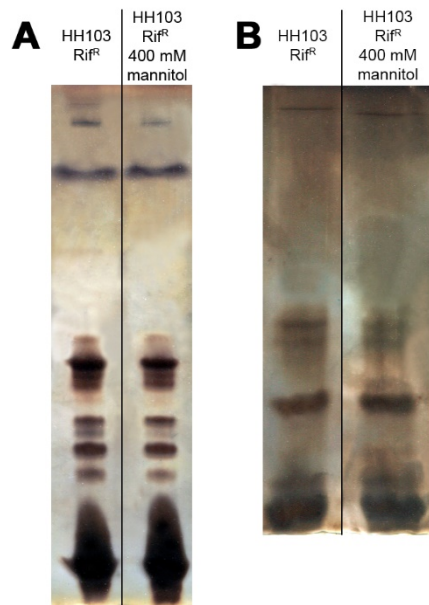
**Supplementary Dataset S4.** List of the different AHLs detected as produced by *S. fredii* HH103 grown in the absence (269) or presence (269\_Manitol) of 400 mM mannitol.



**Figure S1.**  $\beta$ -galactosidase activity of *S. fredii* HH103 carrying plasmid pMP240 upon growth in YM supplemented with different mannitol concentration (from 55 mM to 1 M). Asterisks (\*) indicate significant differences with the corresponding control sample using the non-parametric test of Mann–Whitney,  $\alpha = 5\%$ .



**Figure S2.** Growth curves of *S. fredii* HH103 in different media supplemented or not with 400 mM mannitol. (A) TY medium; (B) YM medium; (C) Minimal medium (MM); (D) Bromfield medium (BF).



**Figure S3.** LPS (**A**) and KPS (**B**) electrophoretic profiles of *S. fredii* HH103 grown in TY medium in the absence or presence of 400 mM mannitol.

**Table S1.** Fold-changes, as determined by RNAseq, of the *S. fredii* HH103 *nodABC* genes after 16-h growth in the presence of 400 mM mannitol.

Gene_name	GENEID	log2BaseMean	log2Ratio	STDERR_log2Ratio	pvalue	padjust	FoldChange
<i>nodA</i>	psfHH103d_126	9.408819572	1.0628198	0.615502891	0.08421252	0.20073394	2.08901058
<i>nodB</i>	psfHH103d_127	9.451582027	0.90654382	0.442138801	0.04032929	0.11817993	1.87454936
<i>nodC</i>	psfHH103d_128	9.235802154	1.4530014	0.448751746	0.00120424	0.00866408	2.73777029