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### A ClpB Chaperone Knockout Mutant of Mesorhizobium ciceri Shows a Delay in the Root Nodulation of Chickpea Plants

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**Abstract:** Several molecular chaperones are known to be involved in bacteria stress response. To investigate the role of chaperone ClpB in rhizobia stress tolerance as well as in the rhizobiaplant symbiosis process, the clpB gene from a chickpea microsymbiont, strain Mesorhizobium ciceri LMS-1, was identified and a knockout mutant was obtained. The ClpB knockout mutant was tested to several abiotic stresses, showing that it was unable to grow after a heat shock and it was more sensitive to acid shock than the wild-type strain. A plant-growth assay performed to evaluate the symbiotic performance of the clpB mutant showed a higher proportion of ineffective root nodules obtained with the mutant than with the wild-type strain. Nodulation kinetics analysis showed a 6- to 8-day delay in nodule appearance in plants inoculated with the Delta clpB mutant. Analysis of nodC gene expression showed lower levels of transcript in the Delta clpB mutant strain. Analysis of histological sections of nodules formed by the clpB mutant showed that most of the nodules presented a low number of bacteroids. No differences in the root infection abilities of green fluorescent protein tagged clpB mutant and wild-type strains were detected. To our knowledge, this is the first study that presents evidence of the involvement of the chaperone ClpB from rhizobia in the symbiotic nodulation process.

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