

NAD-malic enzyme affects nitrogen fixing activity of *Bradyrhizobium japonicum* USDA 110 bacteroids in soybean nodules

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Abstract: The NAD⁺-dependent malic enzyme (DME) has been reported to play a key role supporting nitrogenase activity in bacteroids of *Sinorhizobium meliloti*. Genetic evidence for a similar role in *Bradyrhizobium japonicum* USDA110 was obtained by constructing a *dme* mutant. Soybean plants inoculated with a *dme* mutant did not show delayed nodulation, but formed small root nodules and exhibited significant nitrogen-deficiency symptoms. Nodule numbers and the acetylene reducing activity per nodule as a dry weight value 14 and 28 days after inoculation with the *dme* mutant were comparable to those of plants inoculated with wild-type *B. japonicum*. However, shoot dry weight and acetylene reducing activity per nodule decreased to ca. 30% of the values in plants with wild-type *B. japonicum*. The sucrose and organic acid (malate, succinate, acetate, α -ketoglutarate and lactate) contents of the nodules were investigated. Amounts of sucrose, malate and α -ketoglutarate increased on inoculation with the *dme* mutant, suggesting that the decreased DME and nitrogenase activities in the bacteroids resulted in a reduction in the consumption of these respiratory metabolites by the nodules. The data suggest that the DME activity of *B. japonicum* bacteroids plays a role in nodule metabolism and supports nitrogen fixation.

Author Keywords: *Bradyrhizobium japonicum*; NAD-malic enzyme; Soybean nodules; Symbiotic nitrogen fixation

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