

Characterization of the PotABCD and PotFGHI transporters in *Sinorhizobium meliloti* Rm8530 during free life and symbiosis

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Polyamines are ubiquitous polycations with important but often ill-defined roles in growth, stress resistance, pathogenicity or symbiosis¹. The most common polyamines in prokaryotic and eukaryotic cells are putrescine and spermidine. Although most organisms synthesize polyamines, most also have systems for their transport. Polyamine uptake systems in bacteria include the ATP-binding cassette transporters PotABCD (spermidine-preferential uptake) and PotFGHI (putrescine-specific uptake). The genome of the *Medicago* microsymbiont *Sinorhizobium meliloti* encodes orthologues of *potFGHI* (*smc00770-smc00773*) and *potABCD* (*sma0953-sma0950*). Mutants in either of these systems were grown in succinate-ammonium minimal medium with and without exogenous polyamines. In minimal medium without added polyamines, the *potF* mutant grew like the *S. meliloti* wild type strain Rm8530. However, the *potF* mutant grew poorly when putrescine replaced ammonium as sole nitrogen source in the minimal medium. In minimal medium without polyamines the *potA* mutant showed a marked reduction in growth and lower levels of intracellular polyamines than the wild type and *potF* mutant. Exogenous putrescine did not restore the growth of a *potA* mutant in either the wild type Rm8530 genetic background or in an ornithine decarboxylase 2 (*odc2*) mutant background. The *odc2* mutant makes just trace amounts of endogenous polyamines and should have a greater requirement for their uptake from the environment. Exogenous putrescine did complement the growth of an *odc2* single mutant, indicating the involvement of PotA in putrescine uptake. This was confirmed by analyzing intracellular polyamine accumulation in wild type and mutant cells incubated with putrescine. Both the *potF* and *potA* mutants had significantly lower rates of uptake versus the wild type, demonstrating their participation in transporting this diamine. Symbiosis assays with alfalfa showed a wild type phenotype for plants inoculated with the *potF* mutant. In contrast, plants inoculated with the *potA* mutant were smaller and had lower biomass. We are currently investigating the role these transport systems in the early stages of symbiosis, their relevance to other polyamine-dependent phenotypes in free life, and the further characterization of their polyamine uptake specificities.

References

1. Dunn MF, Becerra-Rivera VA (2023) The biosynthesis and functions of polyamines in the interaction of plant growth-promoting rhizobacteria with plants. *Plants* 2023, 12, 2671.

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