Novel pathways of arsenic detoxification of the legume symbiont *Sinorhizobium meliloti*

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Arsenic resistance genes are ubiquitous in bacteria. In the chromosome of the alfalfa symbiont, *Sinorhizobium meliloti,* is an *arsRSCH* (arsenic resistance) operon. ArsR is a transcriptional regulator, and ArsC is an arsenate reductase. AqpS is a homologue of the *E. coli* GlpF glycerol facilitator, a member of the aquaglyceroporin family of neutral solute channels. We have shown that GlpF and eukaryotic AQPs are $As(OH)_3$ channels, but AqpS is the first aquaglyceroporin whose physiological role is arsenic resistance. In many bacteria, resistance to As(III) is conferred by an efflux protein such as the *E. coli* ArsB $As(III)/H^+$ exchanger. In contrast, AqpS catalyzes only downhill transport. In *E. coli* downhill influx of As(III) by GlpF renders cells sensitive to arsenite. It was anticipated that expression of AqpS would do the same, which raised the question of how it confers arsenic resistance. Our hypothesis is that AqpS functions in arsenate but not arsenite resistance. As(V) is taken up by the Pit phosphate transporter and subsequently reduced to As(III) by ArsC. Internally generated As(III) would then be eliminated from the cell by downhill efflux through AqpS. We find that *S. meliloti* is resistant to extracellular As(V) but is sensitive to extracellular As(III), presumably because it can flow into the cell via AqpS. ArsH is a novel NADPH-dependent flavoenzyme found in some *ars* operons. We find that an *arsH* disrupted strain of *S. meliloti* is As(III) hypersensitive, whereas expression of ArsH in *E. coli* and *S. meliloti* significantly increase As(III) [but not As(V)] resistance, and its action is independent of other *ars* genes. These results suggest that ArsH detoxifies As(III) by a novel redox mechanism independent of transport-mediated pathways. *Supported by a Wayne State University Research Grant Program award to HB and NIH grant GM55425 to BPR.*