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Nitrate reducing commensal bacterium stimulates germination through nitric oxide production-a hypothesis

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The pink-pigmented (PPFM) commensal bacterium, *Methylobacterium extorquens* AM1, stimulates germination of soybean and other plants. I am testing the hypothesis that germination is stimulated by bacterial-produced nitric oxide (NO). A potential source of NO is nitrate reductase (NR) action on nitrite. Our goal was to identify NR gene(s), disrupt it(them) and examine germination stimulation of the mutants. Two nitrate reductase (NR) sequences were identified in the *M. extorquens* genome. Primers were designed and used to amplify both full-length and internal fragments of each NR gene. The internal fragments were cloned into a suicide vector (pAYC61) which encodes PPFM-expressed resistance gene to tetracycline (tetR). Tri-parental mating was then used to introduce pAYC61, carrying the internal NR sequences, into the PPFM strain, and tet-resistant PPFM colonies were selected. Methanol was used as the sole carbon source, thus selecting against the *E. coli* partners in the mating. A screen was devised to test among the tet-resistant progeny "exconjugates" for those with homologous integration of the internal NR sequence integrated into the respective host PPFM NR gene. Such integration will create two non-functional incomplete copies of the host NR gene. The screen will be based on PCR whereby primers will match vector sequence with NR sequence that is NOT part of the internal fragment. Once each NR gene is confirmed to be interrupted, the mutants will be tested for their ability to reduce nitrate to an utilizable nitrogen source. Germination stimulation and seedling root development will be examined by imbibing seed with progenitor and NR- PPFMs. The experiment will confirm or refute our observations of increased germination and lateral root formation in plants with PPFMs. Further, it will indicate whether such effects are due to nitric oxide produced by bacterial NR action on nitrate. Controls will be bacterium grown with without nitrate and seedlings grown with external NO sources and with an NO trap.