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## PPFM to the plant: Just say NO!

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Pink-pigmented commensal bacteria, Methylobacterium spp. are facultative methylotrophs (i.e. they utilize methanol, but they can also use other carbon compounds) commonly referred to as PPFMs. PPFMs associate with seeds and other tissues of soybean and other plants and have been shown by the Polacco lab, and others, to stimulate germination. We tested the hypothesis that germination stimulation was due to production of nitric oxide (NO). One possible source of NO in plants and bacteria is nitrate reductase (NR) which can reduce nitrite (not its "normal" substrate) to NO. M. extorquens AM1 has two forms of nitrate reductase. The first, encoded by the nap gene, is the periplasmic form. The nar gene encodes the respiratory membrane-bound form. Primers were designed to amplify internal fragments of the two nitrate reductase genes, NR1404 (nap) and NR1537 (nar). These fragments were then cloned into a suicide vector (pAYC61), which allows for insertional disruption of the endogenous, nitrate reductase genes. The suicide vector contains a sequence that encodes resistance to tetracycline (tetR). Tri-parental mating was performed among an E. coli strain carrying a mobilization helper plasmid, pRK2013, an E. coli donor strain carrying the suicide plasmid, and the recipient PPFM strain. The availability of the tetR sequence in the suicide plasmid allows screening of the recipient PPFMs that have incorporated pAYC61 into their genomes and growth on methanol as a carbon source selects against E. coli. We will test the NR disruption mutants for NO production, for nitrate utilization, and for the ability to stimulate soybean germination.