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What do pink pigs and soybeans have in common? Selecting for mutants in the ureide pathway

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Pink Pigmented Facultative Methylophilic bacteria (PPFMs) have been found to be the most abundant microorganisms among phylloplane microflora, and have been recovered from all plants examined. PPFMs are seed-transmitted and have been shown to enhance germination. PPFMs may contribute nitrogen, which is an essential nutrient, to the plant. In an attempt to determine this and gain a better understanding of the genes involved in ureide (allantoin, allantoate, etc,) utilization in PPFMs, we tried to elicit mutants along the pathway. We performed a bi-parental mating between the PPFM soybean isolate (wild-type) and an *E. coli* strain containing a plasmid for a kanamycin resistance. This antibiotic resistance plasmid was randomly inserted into the PPFM's genome. The goal is to select mutants that lose the ability to break down ureides. Selection on different media is used to isolate the different mutants along the pathway. Seed surface sterilization does not remove PPFMs since they are found below the seed coat. We have devised a method to eliminate the bacteria by heating the seeds at 50°C for 48 hours. This treatment does not damage the seed. Heat-treated and un-heated soybean seeds were inoculated with a kanamycin resistant soybean isolate strain of PPFM (B140). These plants were grown to maturity in the greenhouse and seeds were collected. We germinated this second generation of seed and are looking to recover kanamycin resistant PPFM bacteria both from the seed as well as the first unifoliate leaves of the soybean. We were able to isolate several putative PPFM ureide utilization mutants. This research will give insight into the interactions between PPFMs and soybean that may be applied to many other plants. Experiments to recover kanamycin resistant bacteria from the seed and from the first unifoliate leaves of the plants are in progress.