ASSESSMENT OF THE STABILITY, FATE AND SURVIVAL OF A ROOT COLONIZING LACZY ENGINEERED PSEUDOMONAS AUREOFACIENS

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Abstract.--A genetic modification of a natural root-colonizing Pseudomonas aureofaciens bacterium was accomplished by inserting into the chromosome two genes from E. coli, lacZ and lacY. The engineered pseudomonad (Ps. 3732RNL11) is now able to utilize lactose as a sole carbon source. This has provided a powerful genetic marker that not only distinguishes the engineered strain from other fluorescent pseudomonads in the environment, but enable an investigator to effectively assess its fate, survival and genetic stability in the field. The natural resistance of the native strain to two antibiotics rifampicin and nalidixic acid, enhances detection, and provides a comparison of the lacZY monitoring system with more traditional recovery based on antibiotic resistance alone. On Nov. 2, 1987, an EPA-approved small-scale field study (< 1.5 acres) was initiated using this engineered strain. During the first planting of winter wheat, approximately 10 CFU OF Ps.3732RNL11 were introduced with the wheat seeds. Root colonization after the first week was about 10 CFU/g root. After three weeks, this level gradually declined to about 10 CFU/g root at harvest, 32 weeks after planting. A second crop rotation of soybean with no additional inoculum was then planted on the field site. Ps.3732RNL11 was found at a low level on the soybean roots during for the first three weeks, but was not detected through the remainder of the soybean growing season (20 weeks). After soybean harvesting, the soil was tilled and a third crop rotation, winter wheat, was planted without additional bacterial inoculum. Analysis of possible re-colonization of the wheat roots by residual Ps. 3732RNL11 in the field soil is now being examined. Movement of Ps.3732RNL11 from the point of initial inoculation was found to be very minimal, detected in rows immediately adjacent to the row inoculated in only 3% of more that 6,500 samples taken. No transfer of the engineered genes in the field to other indigenous microbes has been detected. The presence of the strain has not been observed in the field site plant free area, in water run-off of the field, irrigation pond water, or in/on upper plant parts.