17. Field Release Experiments with Two Genetically Modified Sinor hizobium Meliloti Strains


The Gram- bacterium Sinor hizobium meliloti is common to many soils worldwide. It is of high agricultural importance due to its ability to fix atmospheric nitrogen in symbiosis with the pasture crop alfalfa (Medicago sativa).

In a joint research project about biosafety we focused on the analysis of persistence, spread, and environmental effects of genetically engineered soil bacteria. Therefore, two isogenic S. meliloti strains (GMOs) were marked by chromosomal insertion of a constitutively expressed luc gene of the firefly Photinus pyralis (Selbitschka et al., 1992, 1995). One strain, regarded as wild type, has the luc gene inserted downstream the recA gene and is therefore RecA⁺. The other strain is a RecA⁻ derivative with the luc gene integrated into the recA gene. The RecA⁻ strain is tested for its use as a safety strain in the environment since the recA gene is essential for recombination and repair of DNA. Under laboratory conditions using liquid culture and micocosms an increased sensitivity to DNA-damaging agents, a reduced survival, and a reduced nodulation competitiveness of the RecA⁻ strain compared to the wild type strain were detected (Selbitschka et al., 1992, 1995; Dammann-Kalinowski et al., 1996).

In 1994 and 1995, field release of the two GMOs was carried out at a research site near Braunschweig (Germany). The GMOs were released with a titer of approx. 10⁶ CFU/g soil for the top 25 cm of two model ecosystems, soil columns filled with luvisol (release: September 1994) and small scale field plots (release: April 1995). The luminescence of the GMOs allows to follow their persistence and spread with a detection limit of less than 100 CFU/g soil.

For both systems, a decline of the GMO titer for at least one order of magnitude to approx. 10⁵ CFU/g soil was observed within the first four weeks after release.

In the soil columns, in the following five months during winter time the titer of both strains dropped to approx. 5 x 10⁴ CFU/g soil. During the next six months, the titer of the RecA⁻ strain remained nearly stable whereas the titer of the RecA⁺ strain showed a...
tenfold increase to $5 \times 10^5$ CFU/g soil.

In the field plots, during the first three months after the release (April to June) the titer of the RecA- strain decreased to $3 \times 10^3$ CFU/g soil which is one order of magnitude below the titer of the RecA+ strain with $3 \times 10^4$ CFU/g soil. In autumn, seven months after the release, for both strains the same titer of approx. $1 \times 10^4$ CFU/g soil was measured.

For the analysis of the spread of the released GMOs, we investigated the flow through water of the soil columns. No GMOs could be detected over a period of 13 months, indicating a very limited, if any vertical movement. Due to a low aerosol formation during the release on the field plots, GMOs were detected with a titer of less than $10^2$ CFU/g soil outside the plots. In the non-inoculated control plots, Luc-marked GMOs could be found with a titer of about $10^2$ CFU/g soil and with $10^5$ CFU/g root wet weight in the rhizosphere of alfalah and of a non-host plant. Additionally, we analyzed soil insects collected from the field plots. Only in the feces of a few insects from inoculated plots some GMOs could be found.

When we analyzed the indigenous soil microflora, no indigenous S. meliloti population could be detected before and after the release, presumably due to the fact that the period of fallow before the release exceeded ten months. For the indigenous Rhizobium. leguminosarum bv. viceae population, with a titer of about $10^5$ CFU/g soil no significant changes were detected during the months after the GMO's release. By analysis of the R. leguminosarum bv. viceae strains by PCR-based methods using ERIC and random primers also no effect of the GMOs was found. By application of the BIOLOG GN® system it was also not possible to detect a significant effect on the soil microflora which could be accounted to the GMO's release.

References