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

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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 competitivity 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. 10^6 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. 10^5 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. 5×10^4 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

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tenfold increase to 5 x 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 x 10^3 CFU/g soil which is one order of magnitude below the titer of the RecA+ strain with 3 x 10^4 CFU/g soil. In autumn, seven months after the release, for both strains the same titer of approx. 1 x 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 alfalfa 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

1) Dammann-Kalinowski, T., Niemann, S., Keller, M., Selbitschka, W., Tebbe, C.C. and Pühler, A. (1996): Appl. Microbiol. Biotechnol. **40**: 506-512.
2) Selbitschka, W., Simon, R. and Pühler, A. (1992) : Molec. Ecol. **1**: 1-9.
3) Selbitschka, W., Dresing, U., Hagen, M., Niemann, S. and Pühler, A. (1995) : FEMS Microbiol. Ecol. **16**: 223-232.