3. Evaluation of Possible Horizontal Gene Transfer from Transgenic Plants to the Soil Bacterium *Acinetobacter calcoaceticus* BD413

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The use of genetically engineered crop plants has raised concerns about the transfer of their engineered DNA to indigenous microbes in soil. We have evaluated possible horizontal gene transfer from transgenic field-grown sugar beet (*Beta vulgaris*) or potato plant (*Solanum tuberosum*) by natural transformation to the soil bacterium *Acinetobacter calcoaceticus* BD413. Of the known ways of gene transfer in bacterium, natural transformation (the uptake of naked DNA from the environment) is most likely to mediate horizontal gene transfer from plants. *A. calcoaceticus* can take up DNA fragments independent of their sequence. To be maintained in the bacterium, the DNA needs to be linked to an origin of replication such as via integration into the chromosome or into a plasmid. Several modifications in transgenic plant DNA enhance the possibilities for its stabilization in bacteria. Sequences with homology to bacterial DNA will be present in transgenic plants as the inserted DNA is cloned and maintained in vectors with flanking prokaryotic sequences. Regulatory and protein-encoding sequences from prokaryotic origin are usually also present in transgenic plants. It has been shown that short regions of homology can mediate recombination resulting in addition of non-homologous sequences. Recombination mutants with less stringent homology requirements and illegitimate recombination events are also known to occur.

If the engineered DNA is not transferred to the plant by *Agrobacterium*-mediated gene transfer, integration of DNA into the plant genome will be mediated by electroporation or particle gun systems, both of which typically use intact plasmids with a bacterial origin of replication (*ori*V). Stabilizing sequences inserted into the engineered plant DNA such as *ori*V can bypass the need for homology in the recipient bacteria by plasmid rescue. Thus, possible homology, plasmid rescue, illegitimate recombination events, and recombination mutants can affect the fate of incoming plant DNA in recipient bacteria.

The Gram-negative soil bacterium *A. calcoaceticus* has been shown to be naturally transformable *in vitro*, in water, in soil extract and in soil microcosms. The bacterium is transformable with both chromosomal and plasmid DNA, and most importantly it

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takes up both homologous and heterologous DNA. In previous work (Nielsen *et al.,* 1997), we have optimized the transformation conditions for strain BD413, reaching transformation frequencies of up to 10^{-2} (transformants/recipient (t/r)) with homologous chromosomal DNA. In this study we have focused on how the requirements for homology and *ori*V sequences affected the transformation frequencies. Linearized plasmid DNA (pGSFR 160) without *ori*V, transgenic plant DNA and concentrated transgenic plant DNA, with the selectable *npt* II gene were used. The *npt* II gene was regulated by either the *nos* (potato) or *TR 1-2* (sugar beet) promoter, the latter required rearrangement for expression to occur in *A. calcoaceticus.*

Transgenic plant DNA or linearized plasmid DNA without *ori*V did not give any detectable transformants (detection limit lower than 10^{-11} (t/r)), indicating that homology or a stabilizing sequence like an *ori*V is needed for stable maintenance of transforming DNA. This observation is relevant to the question of possible integration of plant DNA in bacteria as it demonstrates that even after transformation with high numbers of a non-homologous selectable marker gene, expression is not seen in the bacteria.

An *npt* II tagged derivative (chr::*npt* II) of *A. calcoaceticus* BD413 was used as a recipient strain to clarify if sequences of homology between the transforming DNA and the bacterial genome would increase transformation frequencies. An enhanced transformation frequency for the linearized plasmid without *ori*V. of 4.6×10^{-10} was observed. Southern blot analysis revealed integration of the linearized plasmid without *ori*V.

As transformation is affected by competing DNA, a reduced transformation efficiency due to the inhibitory effect of the excess plant DNA will lower transformation frequencies below the detection limit. Transformations with concentrated transgenic plant DNA with the *npt II* gene were used to reduce the effect of competing DNA. Transformants were not detected in these studies either (detection limit better than 10^{-11}) which indicates that the (possible) transformation frequency is less than 10^{-13} (t/r per μg DNA).

Under natural conditions, a further drop of the transformation frequency to at least 10^{-16} is expected due to soil conditions, lower DNA and cell concentration and competition. The results suggest that non-homologous chromosomal plant DNA is practically unavailable for transformation of *A. calcoaceticus* under optimized laboratory conditions. Taken in conjunction with our data on bacterial competence and DNA availability in soil, we conclude that *A. calcoaceticus* is not likely to take up non-homologous DNA in soil at detectable or significant frequencies. If homology is present, horizontal gene transfer could be expected at low frequencies.

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