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Composting rapidly degrades DNA from genetically modified plants

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Organic farmers are concerned about the use of genetically modified plants (GM plants) in conventional agriculture. The concern is mainly focused on the risk of spreading of pollen or seeds from GM plans to adjacent fields.

There has been less focus on the environmental impact of exposing the soil to genetically modified DNA (i.e. transgenic DNA) from GM plants residues left in the field. Yet, the new EU directive on the deliberate release into the environment of genetically modified organisms (EU, 2001) requires a "description of post-release treatment methods for the genetically modified plant material including wastes".

This is based on the fact that there are bacteria in the environment that are able to take up naked DNA. The bacteria mainly take up DNA as a nutrient source, but under certain conditions foreign DNA can be incorporated into the bacterial genome. This mechanism is called horizontal gene transfer.

Until now, horizontal gene transfer from transgenic plants to bacteria has not been detected in natural systems when transgenic plant residues has been allow to decay in soils (Nielsen, 2003). However, the process has been detected in laboratory experiments (e.g., Gebhard and Smalla, 1998). On this background we set up additional experiments in the **CRUCIAL-project** to investigate if composting is a useful method for the elimination of transgenic DNA and thereby produce a bio-safe natural fertiliser from GM plant residues.

Different elements were investigated: i) the persistence of transgenic and wildtype DNA during composting of GM plant residues as compared to incorporating the residues into the soil, ii) the risk of naturally occurring bacteria (*Bacillus*, which is known to become dominant in compost) taking up and incorporating transgenic DNA during composting.

Composting of GM plants

Composting was performed at KVL in a laboratory composting system with PC-controlled compost-reactors **(figure 1)**. The GM plant mixture consisted of dry barley straw and transgenic *Arabidopsis* transformed with the Sorghum gene for CYP71E1 via the Ti plasmid in *Agrobacterium tumefaciens* (the *Arabidopsis* plants were kindly provided by Prof. B. Lindberg Møller). The entire plant including the root of 5 weeks old Arabidopsis plants were used. Both straw and plant were cut into 2 cm pieces. The straw was re-wetted prior to mixing. The final mix had a water content of 86%.

Composting of GM plants were performed in litterbags (1 mm mesh size; 6.7 g/bag) which were placed in compost-reactors filled with a non-GM composting mixture (barley straw and white clover (I) or sugar beet leafs

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(II)). GM composting was performed at two separate occasions. Samples were taken at intervals (Compost I up to 111 days, Compost II up to 77 days) by harvesting an entire litterbag. To simulate ploughing down of the plant material a litterbag with 20 g of the GM plant mixture was kept in a bucket of soil at 17°C. Samples from this were taken until day 77. The presence of both transgenic and wildtype DNA was detected by DNA purification and PCR.

Rapid DNA degradation

In Compost I the temperature peaked at 58°C and the transgenic DNA could no longer be detected after 10-14 days (for one of triplicate samples, see **figure 2**).

In Compost II the maximum temperature was 68°C resulting in a faster decay of DNA which was no longer detected after 6-10 days **(figure 2)**. In both composts the rate with which the transgenic DNA disappeared was much faster than the experiment where the plant material was kept in soil. Transgenic DNA was still detected after 77 days in the soil experiment **(figure 2)**.

No DNA uptake by *Bacillus*

To determine if *Bacillus* were incorporating transgenic DNA during composting, all other bacteria had to be eliminated from the compost before spreading dilutions onto growth media. Boiling the compost leaving only the spore-forming *Bacillus* to survive can do this. *Bacillus* rapidly became dominating in the compost increasing in numbers from 10^3 to 10^7-10^8 per g compost.

Bacillus was screened for the presence of transgenic DNA by scraping colonies off the growth media, purifying the DNA and running a PCR. In several cases these screenings indicated that *Bacillus* contained transgenic DNA from *Arabidopsis*. This lead to the isolation of 300 colonies which were tested by PCR for the presence of transgenic DNA.

Of these, three isolates gave PCR products of the exact same size as the control DNA, but sequencing of these products revealed that they were not identical to the transgenic DNA. One sequence had highest homology with a *Bacillus halodurans* (one half of the sequence had almost 100% homology whereas the other half had no known homology), the two other isolated were identical and had 98% homology to *Bacillus subtilis*.

Conclusions and further studies

The experiments show that composting of GM plant residues greatly increases the rate of degradation of transgenic DNA compared to the rate for plant residues left in the soil. If this is considered as the only risk factor, composting is a 'DNA-safe' method to treat GM plant residues.

However, even though transgenic plant DNA was not detected in bacterial isolates in our experiments, we cannot conclude that horizontal gene transfer can not take place. The 300 isolates investigated proved to be too low a number to be conclusive.

The numbers of isolates tested were based on the screenings indicating high transfer, but the screenings were biased apparently because some *Bacillus* species gave PCR products matching the transgenic DNA. Thus, it is still an open question if composting constitutes a safe way of disposing of GM plant residues. Furthermore, these experiments give rise to other interesting questions, e.g., whether GM plant materials decomposing in waste piles or deposited in manure yards may be able to transfer genes to

indigenous bacteria at the comparably lower temperatures present at these environments.

These questions need to be assessed if the risk associated with the use of GM plants is to be thoroughly investigated.

References

EU. 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms.

Gebhard, F. and Smalla, K. 1998. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Applied and Environmental Microbiology, 4: 1550-1554.

Nielsen, K.M. 2003. An assessment of factors affecting the likelihood of horizontal gene transfer of recombinant plant DNA to bacterial recipients in the soil and phytosphere. In: Collection of Biosafety Reviews, Vol. 1 pp. 98-149. International Centre for Genetic Engineering and Biotechnology (ICGEB). 2003. Printed by Editoriale Ergon.

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