Measuring degradation of transgenic DNA and screening for horizontal gene transfer from GMO-plant material during composting

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Introduction

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 [3]. However, the process has been detected in laboratory experiments (e.g., [2]). On this background we set up additional experiments in the Short Circuit / CRUCIAL- project domain to investigate if composting is a useful method for the elimination of transgenic DNA and thereby produce a bio-safe natural fertilizer from GM plant residues.

Different elements were investigated: i) the persistence of transgenic and wildtype DNA during composting of GM plant residues (Arabidopsis plants genetically modified with a Sorgum gene) 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.

Rapid DNA degradation

The results of the degradation experiments are shown in fig. 1. In Compost I the temperature peaked at 58°C and the transgenic DNA could no longer be detected after 10-14 days

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. 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.

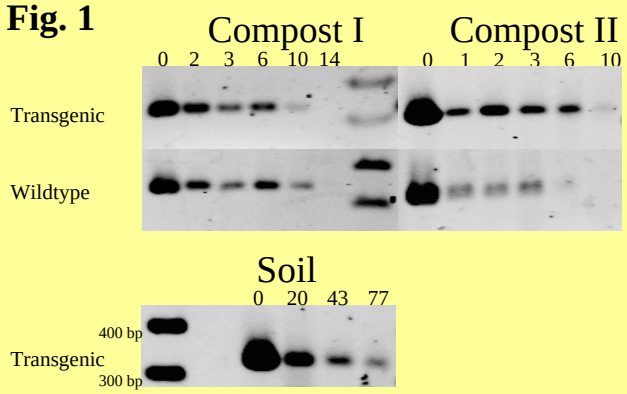
No detection of DNA uptake by Bacillus

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. This was accomplished by boiling compost samples leaving only the spore-forming *Bacillus* to survive. *Bacillus* rapidly became dominating in the compost increasing in numbers from 10^3 to 10^7-10^8 spores 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 led 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*.

Fig. 1



The presents of DNA was investigated by PCR on purified total DNA using primers specific for either transgenic or wildtype DNA. (numbers indicate sampling day)

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 the persistence of transgenic DNA in the environments 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 tested 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., the behavior of GM plant-materials decomposing in waste piles or manure yards under composting-like conditions and the possibility of horizontal gene transfer 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

[1] 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.

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

[3] 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.

The Short-Circuit / CRUCIAL project domain

The Short-Circuit project is a EU-Life funded activity with the aim of ”short-circuiting” the carbon and nutrient cycle between urban and rural districts by establishing three new systems for source separation, collection and composting of organic waste in the greater Copenhagen area. This activity is co-funded by the Danish Research Centre for Organic Farming, via the CRUCIAL project.

These projects form part of a coherent effort to re-invent urban waste management with the view to close the rural urban nutrient cycle. They have ensured the establishment of a field-scale facility for assessing the feasibility of improved recycling of nutrients from urban areas to organic farms, in the form of a long-term field trial. Emphasis on urban fertiliser pre-treatment, turnover in soil and impact on crop growth, will provide practically useful results. With the initiation of a monitoring programme for biological soil quality eventual unforeseen ill effects of increased re-circulation will be taken into account. Additionally it will provide support to planned research on human and animal health aspects, in connection with agricultural waste utilisation. Finally the work provide at concrete platform for the public debate, and possibilities for the public to visit the field site. For more information refer to www.nutrap.dk

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