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Microbial and nematode communities associated with potatoes genetically modified to express the antimicrobial peptide magainin and unmodified potato cultivars

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Abstract

The antimicrobial peptide magainin II has activity against a range of micro-organisms. Tubers harvested from potatoes genetically modified (GM) to express a synthetic magainin gene show improved resistance to the bacterial pathogen *Erwinia carotovora*. The microbial and nematode communities associated with three magainin-expressing potato lines, their near-isogenic, unmodified parental cultivar (Iwa) and an unrelated cultivar (Karaka) were investigated on field-grown plants. Heterotrophic plate counts were used to enumerate aerobic culturable bacterial and fungal populations, while cultivation-independent analysis of bacterial communities was based on denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments amplified from community DNA from phyllosphere, rhizosphere and geocaulosphere (tuber surface) samples. Small but statistically significant differences in the population sizes of culturable bacteria, fungi and yeast were detected among some GM magainin-expressing lines and the unmodified control. However, these differences were typically smaller than the differences between the unmodified parental line control (Iwa) and the unrelated cultivar control (Karaka). Similarly, the difference in the proportion of the nematode population belonging to the fungal feeding trophic group between Iwa and Karaka was greater than that amongst Iwa and its near-isogenic GM lines, and was significantly so for the genus *Aphelenchus*. The nematode channel ratio (NCR) indicated a more fungal-dominated decomposition channel in soil beneath Karaka compared to Iwa at harvest. In general, eubacterial phylloplane communities were similar for all lines, while the rhizosphere communities associated with two of the three GM lines differed from communities associated with their unmodified parental line control. When roots were senescent, there was no significant difference among potato lines in rhizosphere eubacterial communities or individual trophic groups of the nematode community. Greater diversity was found in geocaulosphere; α-proteobacteria and actinomycete communities of two of the three GM lines differed significantly from their unmodified parental line control and the unrelated cultivar control, while the communities associated with the third GM line were more similar to those of the two control lines. This highlights the importance of testing several GM lines when assessing non-target effects. Results suggest that there is little likelihood of any major sustained non-target effect of genetic modification using a magainin II transgene on plant-associated and soil microflora and function.

Keywords: Genetically modified potato; Antimicrobial peptide; Magainin; Bacterial diversity; Nematode community analysis; 16S rRNA; Phyllosphere; Rhizosphere; Geocaulosphere

1. Introduction

Potatoes have been the focus of many genetic modification programmes, in particular incorporating resistance to

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bacterial and fungal diseases. Genetic modifications of potatoes for broad-spectrum disease resistance include transgenic expression of enzymes such as T4-lysozyme (Düring et al., 1993), glucose oxidase (Wu et al., 1995) and oxalate oxidase (Schneider et al., 2002); plant proteins (Gazendam et al., 2004); tachyplesin I (Allefs et al., 1996); and the lytic peptides cecropin B (Arce et al., 1999) and dermaseptin B1 (Osusky et al., 2005).

There are limited data on the effects of introducing genes for production of antimicrobial substances into plant genomes. Some of the antimicrobial substances have activity against a broad range of bacteria and fungi and could potentially affect natural plant-associated bacterial and fungal populations, e.g. plant-growth promoting bacteria found in the plant rhizosphere. Some genetically modified lines of potato have been examined for non-target effects. The effects of T4-lysozyme-producing potatoes on plant-associated bacteria were reported to be minor in comparison with the natural variability observed during the monitoring periods (Lottmann et al., 1999, 2000; Heuer et al., 2002). Sessitsch et al. (2003) showed that at the flowering stage, cecropin-expressing lines caused a transient but significant effect on the diversity and community structure of culturable *Bacillus* spp. Community structures on modified potato lines carrying only vector sequences showed diversity values comparable to the *Bacillus* communities found in rhizospheres of wildtype plants. Little difference was seen in *Bacillus* communities at tuber production stage. More recently, results from a greenhouse experiment examining rhizosphere bacteria associated with potato lines expressing cecropin or T4-lysozyme grown in two different soil types demonstrated that the impact of genetic modification was transient and minor, and differences in parameters measured were comparable to natural variation caused by soil type, vegetation stage at time of sampling and pathogen exposure (Rasche et al., 2006a).

Magainin II is an antimicrobial peptide isolated from the skin of the African clawed toad (*Xenopus laevis*). It has shown activity *in vitro* against a range of micro-organisms, including some species of Gram-negative and Gram-positive bacteria (Giacometti et al., 1998) and bacterial plant pathogenic species including *Pseudomonas* spp., *Erwinia* spp. and *Clavibacter* sp. (Alan and Earle, 2002). The yeast species *Saccharomyces cerevisiae* (Zasloff et al., 1988) and various plant-colonising and pathogenic fungi, including *Penicillium*, *Alternaria* and *Phytophthora* spp. (Alan and Earle, 2002; Jacobi et al., 2000; Kristyanne et al., 1997), have also shown sensitivity to synthetic magainin peptides. Tobacco and banana plants genetically modified to produce a magainin analogue MSI-99 showed enhanced resistance to a range of fungal pathogens, including *Sclerotinia*, *Alternaria*, *Fusarium* and *Botrytis* spp. (Chakrabarti et al., 2003). Bioassays of potato tubers produced by magainin-expressing potato plants against *Erwinia carotovora* showed less damaged tissue than unmodified control tubers (Barrell, 2001), indicating that sufficient magainin II was produced in the tubers to control *Erwinia* infection. Within these plants, magainin II is secreted into the intercellular spaces in the plants and has the potential to change the structure of the microbial community associated with the leaves, roots and tubers.

A preliminary study on a small-scale field trial of magainin-expressing potatoes examined culturable bacterial and fungal populations associated with a magainin-expressing potato line and the unmodified parental line control (O'Callaghan et al., 2004). Culturable bacterial populations recovered from the leaves and roots of the plant lines did not differ significantly. However, tubers from the magainin-expressing transgenic potatoes had significantly lower populations of Gram-positive bacteria than tubers produced by unmodified plants. There were no significant differences in the total numbers of fungi and yeasts recovered from the various plant lines, with one exception: higher numbers of fungi were recovered from roots of magainin-expressing plants than the unmodified control plants.

The aim of the present study was to characterise in greater detail the bacterial and fungal communities associated with three lines of magainin-expressing potatoes, an unmodified parental line control and an unrelated cultivar control. In addition to enumeration of culturable populations, culture-independent analysis of the bacterial communities was undertaken by DNA extraction from phyllosphere, rhizosphere and geocaulosphere samples, followed by PCR-DGGE (PCR-denaturing gradient gel electrophoresis) analysis of the rRNA gene fragments. Soil nematode communities were also sampled at two times during the growing season as biological indicators of soil biota.

2. Materials and methods

2.1. Plant material and field trial

Transgenic and non-transgenic potato plants were sampled from a field trial in the 2002/2003 southern hemisphere growing season at the New Zealand Institute for Crop and Food Research Limited, Lincoln, Canterbury, New Zealand (Environmental Risk Management Authority approval GMF98007). The potato lines D2, D5 and D9 were derived from *Solanum tuberosum* cv. Iwa using *Agrobacterium*-mediated transformation with a binary vector containing a kanamycin-resistant selectable marker gene, plus a synthetic magainin II gene under transcriptional control of a 35S promoter and with a translational fusion to a signal sequence to direct export of the magainin II peptide into the intercellular space (Barrell, 2001). The lines D2 and D5 had single inserts of the transferred DNA. Both were confirmed as producing correctly processed magainin II by western analysis on leaf samples, indicating successful export out of potato cells, with D2 showing only low protein accumulation and D5 high protein accumulation (Barrell, 2001). Line D9 possessed six copies of the transferred DNA and although