Factors influencing transgene survival and transfer in the rumen

Area of research interest: Novel and non-traditional foods, additives and processes
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Background

This study addressed the possibility that the transfer of genes may occur from transgenic food/feed to gut microbes. Transgenic plant material, particularly genetically modified (GM) maize, is already used widely in animal feedstuffs in many parts of the world. Many more experimental GM plant lines are being explored by plant biotechnologists that might offer a variety of agronomic, health or nutritional benefits in the future, and that might therefore be utilised as feed components. The potential for gene transfer therefore needs to be considered with respect to the microbiota inhabiting the gastrointestinal tract of farm animals.

Research Approach

In vitro modelling of the rumen, together with real-time polymerase chain reaction (PCR) detection of specific gene sequences were used to examine the release and survival of DNA during the degradation of GM plant material. In addition, model transgene constructs carrying multiple selectable marker genes were used to study factors that influence acquisition of external DNA by the gut microflora, including antibiotic treatment and sequence homology (similarity) with resident genes. The marker genes allowed selection of transformed gut bacteria, and their recovery from metagenomic libraries. Any gene transfer detected was rigorously confirmed by analysis of flanking sequences.

Results

Key Results

Most of the variables tested had no significant effect on the survival time of DNA in rumen fluid. The observed half-life for detection of DNA sequences from maize and soya plant material following exposure to rumen fluid was 4-6h, while rapeseed was degraded a bit more rapidly and had a half-life of about 3h. The only variable that significantly affected DNA survival was the presence or absence of protozoa in the rumen fluid. These eukaryotic microbes engulf plant material, and prolonged the time the GM sequences could be detected.
When the plant material was incubated under simulated rumen conditions, transgenes were detected in the solid fraction – residual plant material – 24h after incubation, in those vessels where GM soya and maize had been added. However transgenes were never detected in the bacterial fractions extracted from either the liquid or solid-associated bacterial phase, indicating that there was no detectable uptake of GM DNA from plant material by rumen bacteria.

In the final experiment specific DNA constructs, designed to contain detectable marker genes and regions of bacterial DNA, were added to simulated rumen conditions and the persistence of the DNA followed. The model transgenes were taken up by a sub-population of the rumen bacteria, at a low level. The results indicated that linear GM DNA could be taken up by naturally transformable commensal rumen bacteria – if bacterial DNA sequences are associated with the transgene. A range of bacterial species acquired, incorporated and expressed the plasmid DNA. The number of DNA uptake events was increased when antibiotic selection pressure was applied, but were still so rare that they could not be analysed statistically, using standard methods.

Conclusions

The main findings from this work were that GM DNA sequences can be detected within residual GM plant material that has been exposed to rumen fluid in vitro, even after 24h. However these sequences were never detected in any of the rumen bacteria using rt-PCR. Rumen bacteria were only able to acquire GM DNA if there was a region of bacterial DNA associated with the transgene, to facilitate homologous recombination events. Even then, the uptake of DNA by rumen bacteria was an extremely rare event.

Published Papers

1. Minas, K., Mcewan, N.R., Newbold, C.J. & Scott, K.P. (2011) Optimization of a high-throughput CTAB-based protocol for the extraction of qPCR-grade DNA from rumen fluid, plant and bacterial pure cultures. *FEMS Microbiology Letters* 325: 162-169

Research report

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