

Section 2.1 : Non-Technical (Layperson's) Executive Summary

Project Title: Survival of ingested DNA in the gut and the potential for genetic transformation of resident bacteria

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There is increasing interest in Genetically-Modified (GM) plant material and microorganisms as means to improve both food quality and the efficiency of food production. This project was designed to address scientific and public concerns over the fate of GM DNA in the human gut following ingestion. To this end we investigated the survival of ingested DNA *in vitro* under simulated gut conditions and *in vivo* in the rat gut, and also the potential for genetic transformation of resident oral and gut bacteria by surviving DNA. This research would therefore provide the public and the Food Standards Agency (FSA) with important information regarding the survival of GM plant DNA and GM micro-organism survival under gut conditions, and may be used to inform the safety assessment of GM foods carried out on behalf of the FSA in the UK by the Advisory Committee on Novel Foods and Processes (ACNFP).

The survival of DNA in the human mouth was determined *in vivo* by a human volunteer, after receiving the necessary ethical committee approval. The half-life of DNA *in vivo* was only 6 seconds and the concentration had decreased ~ 100-fold after only 60 seconds exposure. DNA was much less stable under *in vivo* conditions than was apparent from previous *in vitro* experiments with human saliva. Nevertheless, when tested *in vitro*, sufficient partially degraded DNA survived to bring about genetic transformation, (uptake of free DNA and its heritable incorporation into the bacterial genome) to antibiotic resistance, of a strain of the oral bacterium *Streptococcus gordonii*. Since *S. gordonii* is capable of transformation in human saliva without any special pre-treatment, the possibility must exist for transformation of related bacteria to occur *in vivo*. In the course of this work we established that a lower gut strain of another Streptococcus species, *S. bovis*, which has been implicated in disease causation, was similarly capable of natural transformation. Transformation of both *S. gordonii* and *S. bovis in vitro* was observed in saliva, but not under simulated small intestinal or colonic conditions. Selected strains of *Escherichia coli* (ubiquitous throughout the gut) and *Helicobacter pylori* (gastric bacteria) can be transformed *in vitro*. We were unable to transform gut isolates of these bacteria under conditions simulating those of the mouth or stomach. DNA survival will of course vary with different gut conditions. *In vitro* data obtained here identified some food components that may enhance DNA survival.

In most cases, fragments of GM DNA in food will not be capable of propagating themselves in a gut or oral bacterium even if they are taken in by bacterial cells. They are only likely to become heritably acquired if they insert into the host chromosome. This could occur, if the GM DNA and bacterial chromosome share regions with identical sequences. The transformation of two native gut bacteria, *S. gordonii* and *S. bovis*, was therefore investigated using DNA that is able to integrate into the bacterial chromosome as a result of matching sequences provided by an antibiotic resistance marker gene. Higher transformation efficiencies were obtained *in vitro* with DNA that was able to integrate into the bacterial chromosome than with self-replicating plasmid DNA that had no sequence match with the bacterial chromosome. We did not detect transformation *in vitro* using linear DNA that possessed only a single region of matching sequence, which is, arguably, the most likely state for GM DNA in food. We did, however, detect transformation for genes that were flanked on both sides by sequences that match the bacterial chromosome.

When naked GM DNA was fed to rats, DNA from marker genes remained detectable in the rat faeces for up to 79 hours. Preliminary data indicated the possibility that transformation of gut bacteria by the added GM DNA might occur in the rat gut, but rigorous confirmation is lacking. In order to study the fate of GM bacteria under gut conditions, marked strains were added to fermentor simulations of the human colon, or fed to rats. GM *Lactococcus lactis* were rapidly eliminated from the fermentor, suggesting active killing by the resident microflora, but for a tail population that was able to establish itself in the fermentor at low numbers. This tail population was attributed to adaptation of the introduced strain rather than to gene transfer events. GM *Enterococcus faecalis* were fed to laboratory rats and were able to survive in the gut for 11-13 days. During this time no evidence of transfer of the GM marker genes to the native microflora of the rat gut was observed.

In conclusion, there is a possibility of rare acquisition of GM sequences by resident bacteria in the mouth or gut. The probability of such events is influenced by the design of GM constructs since it depends largely on the presence of matching sequences in the host bacterium and GM DNA. This conclusion must however be put firmly into its evolutionary context. Humans have consumed huge amounts of DNA in food throughout evolutionary history, and the possibility of gene acquisition by resident oral and gut bacteria has always existed.