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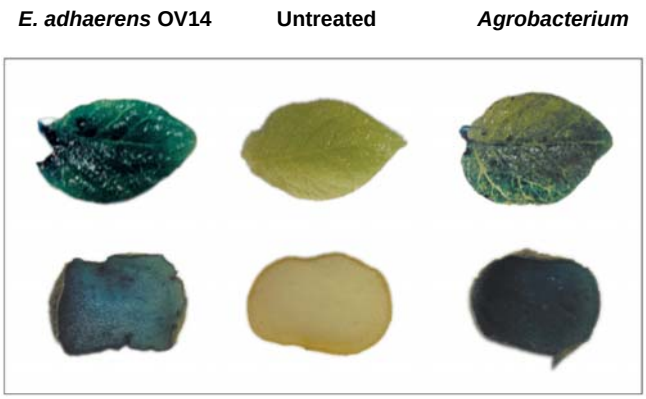
# A novel method for the genetic transformation of plant cells




Genetic transformation of potato leaf (upper) and tuber (lower) tissues with *E. adhaerens* OV14 compared to *Agrobacterium.* Blue staining indicating the presence of transformed tissues.

## Key external stakeholders:

Plant scientists from the crop biotech industry, universities and private research institutions working on the application of genetic engineering to improve the agronomic potential of existing plant varieties.

## Practical implications for stakeholders:

The primary technique for the generation of engineered or 'biotech' crops utilises the bacteria *Agrobacterium tumefaciens* in a process used worldwide by public-sector agencies, private industries and universities. Yet to the end user, trying to commercialise a product developed through *Agrobacterium*-based technology is not feasible due to a series of key patents on transformation technology. Output from our research has:

- Identified a novel bacterium *Ensifer adhaerens* OV14 that will successfully transfer single/multiple gene(s) of interest into plant cells at rates equivalent to standard *Agrobacterium*-based technology.
- Noted that *E. adhaerens* OV14 is genetically distinct from *Agrobacterium* and as such circumvents existing transformation patents on plant species but with no loss of efficacy.
- Discovered that *E. adhaerens* OV14 does not require challenging conditions or processes for its growth and can be integrated into existing *Agrobacterium*-based technology with no additional optimisations required.

## Main results:

- A novel transformation platform has been developed around the bacterium *E. adhaerens.*
- Using *E. adhaerens* circumvents existing patent restrictions on the genetic transformation of plant species.
- Ensifer-mediated transformation produces equivalent rates of transformation to the existing Agrobacterium-based system

## Opportunity / Benefit:

Utilising *E. adhaerens* OV14 will provide companies and/or institutions working on plant biotechnology with the opportunity to work with an equivalent market alternative to the patent restrictive *Agrobacterium*-based technology, which will not require additional end-user training, above what is traditionally required for *Agrobacterium*-based technologies.

## Collaborating Institutions:

UCD



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**External collaborators:** Dr. Fiona Doohan, University College Dublin

## 1. Project background:

The current challenges in biological research are to understand how genes function together in metabolic pathways so that real world problems can be addressed, ranging from improving crop yields to generating varieties that produce novel fuels and/or high value products. Disrupting the function of a specific gene through random and/or precise modifications has made it possible to address many of these challenges. This process of gene mutagenesis can be completed through several techniques but the bacteria *A. tumefaciens* is the favoured tool for genetic engineering.

A soil inhabiting bacteria, *A. tumefaciens* is a natural pathogen of plants causing 'crown gall' disease across a broad host range. *A. tumefaciens* is able to infect plants by effectively shuttling specific genes of interest into a target cell, where in they are switched on. Scientists have capitalised on this phenomenon and equipped *A. tumefaciens* with genetic machinery that makes the process efficient and reproducible.. As a result *A. tumefaciens* has underpinned the development and commercialisation of biotech crops. Since their commercialisation in 1997 the global acreage of licensed biotech crops has increased annually; 104 million hectares were grown in 2009 with an estimated global market value in excess of $7.5 billion.

However, the application of *A. tumefaciens* is comprehensively covered by patents that have placed a stranglehold on transformation technology. Hence the potential for end-users of *A. tumefaciens*-based transformation to develop commercially viable products/varieties/technologies is severely curtailed due to the comprehensive licensing structure that is in place. So while multiple examples exist detailing the development of novel varieties through *A. tumefaciens*-based transformation, this has not translated in to an increased number of products for stakeholders as patent holders continue to restrict competition.

In an attempt to design around the patent issue the potential of non-*Agrobacterium* strains was described in 2005. Using *Sinorhizobium melliloti*, *Mesorhizobium loti* and *Rhizobium* NGR 234 (collectively called Transbacter™), it was demonstrated that these non-*Agrobacterium* strains could be used to genetically engineer plant cells. However, the transformation efficiency of these Transbacter™ strains was poor compared to standard *A. tumefaciens*-based transformation and their integration into research programmes has been nominal.

## 2. Questions addressed by the project:

- Can we develop a method to isolate from the roots of crop plants a collection of soil-borne bacteria?
- Can this collection of plant-associated bacteria be screened for individuals with the capacity to genetically transform plant cells?
- Can we comparatively assess any positive bacteria strains against the existing transformation systems (*A. tumefaciens* and Transbacter™)?

## 3. The experimental studies:

The bacteria library was generated by washing the root systems of commercially grown plants and spreading this wash suspension on selective microbiological media. The collected bacteria strains were then characterised to determine their suitability for genetic transformation studies. Each bacteria isolate was tested for their:

(i) capacity to take up large pieces of DNA containing 'virulence' genes, which drive the genetic transformation process
(ii) ability to remain viable after long periods of storage (~6 months)
(iii) resistance/susceptibility to specific antibiotics.

The latter was important as antibiotic resistance is a trait commonly used in plant transformation protocols to facilitate the rapid screening of large plant populations for those individual plants that have been genetically modified. Quite simply, a specific gene conferring resistance to an antibiotic (e.g. *hph*, resistance to hygromycin) is attached to the gene of interest and both are transferred into the target plant cell. So if an emerging plant has the capacity to grow in the presence of the antibiotic at concentrations that would otherwise be toxic it must be successfully transformed.



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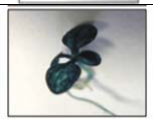
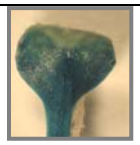
The small cohort of bacteria strains that graduated through this criteria-based selection process underwent sequence-based identification. This was achieved by reading the code of the 16S rRNA gene for each bacteria and comparing each sequence read against that of *A. tumefaciens* and the three *Transbacter*™ strains.

## 4. Main results:

A total of 320 bacteria strains were isolated using the root wash protocol. From this population only four strains graduated for sequence-based identification after which each was tested for their ability to transform the model plant species *Arabidopsis.* Only one of the four strains was successful in this regard. Identified as *Ensifer adhaerens* OV14, this bacterial strain had the capacity to genetically transform the important food and non-food crops, potato and tobacco respectively (Table 1), at rates equivalent to standard *A. tumefaciens*. Significantly, the transformation efficiency of *E. adhaerens* OV14 was 6-fold greater than the best reported Transbacter™ strain and was equivalent to that of *A. tumefaciens*; thereby making *E. adhaerens* OV14 a viable alternative to existing transformation platforms.

Table 1: Transformation of *S. tuberosum* (leaf), *N. tabacum* (seedling) and *Arabidopsis* (leaf) tissues with *E. adhaerens* OV14 and *Agrobacterium* strain LBA4404 as indicated by blue staining of treated tissues compared to untreated control tissues.

| | | Bacteria treatment | | |
|---|---|---|---|---|
| | | *E. adhaerens* | Untreated | *Agrobacterium* |
| Plant species targeted for transformation | *S. tuberosum* (potato) |  |  |  |
| | *N. tabacum* (tobacco) |  |  |  |
| | *Arabidopsis* |  |  |  |

## 5. Opportunity/Benefit:

To protect the licensing potential of this technology a patent was submitted in December 2009 detailing the isolation and characterisation of *E. adhaerens* OV14. Interest has been expressed from both industrial and academic sources, in obtaining a license to use *E. adhaerens* OV14 for specific purposes. In partnership with the technology transfer service within University College Dublin, information detailing the commercialisation potential of utilising *E. adhaerens* OV14 over *A. tumefaciens* is available at www.teagasc.ie.

## 6. Dissemination:

### Main publications:

Wendt T., Wincklemann D., Doohan F. and Mullins E. (2010). Gene transfer into *Solanum tuberosum* via *Rhizobium* spp. *Transgenic Research*, Vol. 20, No.2, p377-386.

Mullins E. (2009). A method for transforming plant cells. Patent submitted to European Patent Office (No. EP09180700.8) and US Patent Office (No. USSN61/289,853), submitted December 23rd.

Wendt, T., Doohan, F. and Mullins, E. (2011). Ensifer-Mediated Transformation: A Novel Platform for the Transformation of Plant Genomes. Presentation to the International Plant Transformation Technology Conference, Vienna, February 19th – 23rd.

## 7. Compiled by: Dr. Ewen Mullins



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