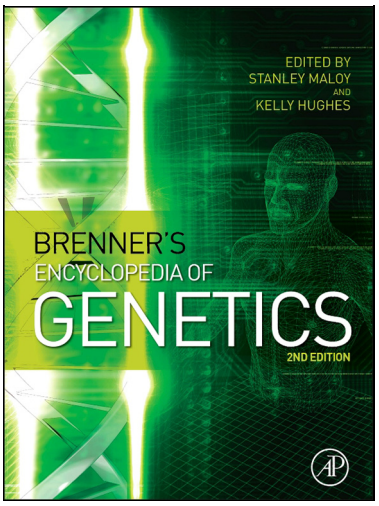

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Agrobacterium

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Glossary

Crown gall Neoplastic growth (tumor) resulting from uncontrolled cell proliferation induced by *Agrobacterium tumefaciens*, usually at the interface between the root and the stem of the host plant.
Hairy roots Uncontrolled root proliferation induced by *Agrobacterium rhizogenes* in its host plants.
Neoplastic growth A mass of uncontrollably proliferating cells not coordinated with the surrounding normal tissue.
Opines Small molecules composed of an amino acid and a keto-acid or a sugar.
T-DNA Transferred DNA, segment of DNA on the tumor-inducing (Ti)-plasmid delimited by two short tandem repeat sequences (borders), that is transferred to host cell during *Agrobacterium* infection process.
Ti-plasmid Tumor-inducing plasmid; large plasmid present in phytopathogenic *Agrobacterium* strains, harboring the T-DNA region (responsible for gall formation and opine production) and the virulence region comprising genes required for T-DNA transfer.
Ri-plasmid Root-inducing plasmid; large plasmid present in phytopathogenic *Agrobacterium rhizogenes* strains, harboring the T-DNA region (responsible for root proliferation and opine production) and the virulence region comprising genes required for T-DNA transfer.

General Properties of Agrobacteria

Morphology

Agrobacteria are a group of Gram-negative, non-spore-forming soil bacteria, often isolated from abnormally proliferating plant tissues. These motile (containing up to six flagella per cell), aerobic (using oxygen as a final electron acceptor during cellular respiration), rod-shaped bacteria have a rather slow (from one to several hours) generation time even under the optimal laboratory conditions. Agrobacteria are able to catabolize a large variety of metabolites. They exhibit chemotactic behavior for some plant exudates, which they use in nature to initiate colonization of a susceptible plant tissue.

Taxonomy

The genus *Agrobacterium* belongs to a large family of plant-associated bacteria, termed *Rhizobiaceae*, which also include nitrogen-fixing symbiotic bacterial genera such as *Rhizobium* and *Sinorhizobium*. Over the years, analyses based on new taxonomic criteria, including 16S RNA sequence comparisons and, more recently, complete genome sequences of some strains, pointed toward the close relationship of these genera. In fact, they are so close that some taxonomists question the relevance of the discrimination between *Agrobacterium* and *Rhizobium*, and have proposed to abolish this distinction and to regroup these species under the genus name of *Rhizobium*. However, the name *Agrobacterium* remains in use in most of soil microbiology, plant physiology, and plant molecular and cell biology publications and applications.

Traditionally, the classification of different species of *Agrobacterium* was based on their phytopathogenic properties. Initially, three species were described: *A. tumefaciens*, capable of inducing crown galls on a large spectrum of dicotyledonous plants; *A. rhizogenes*, which induces the hairy root disease; and the non-phytopathogenic strain, *A. radiobacter*. Later, other *Agrobacterium* species were distinguished that had a very limited host range and induced plant cell proliferation only in some plant species. This was the case with *A. vitis*, specific for grapevine, *A. rubi*, rather specific for some *Rubiaceae*, and *A. larrymoorei* isolated from *Ficusbenjamina*. This classification, however, became invalid when phytopathogenicity and/or the host range of Agrobacteria was demonstrated to arise from the presence of large transmissible plasmids, termed 'Ti (tumor-inducing) plasmids' for *A. tumefaciens* and 'Ri (root-inducing) plasmids' for *A. rhizogenes*, whereas the bacterial strain cured of these plasmids was found to represent *A. radiobacter*. With the advent of genome sequencing and new methodologies for studies of metabolic pathways (metabolite displays), different *Agrobacterium* isolates were grouped into three taxonomic clusters or biovars (Table 1), which, ultimately, could be considered genera.

Genome Structure

Presently, the complete genome sequences are available for representatives of all three *Agrobacterium* biovars. These data revealed that each of these biovars is distinguished by a specific composition of its genome (Table 1). For example, the genome of *A. tumefaciens* strain C58 (biovar I) comprises a 2.8-Mb circular chromosome, a 2-Mb linear chromosome, a 214-kbp pTi58 plasmid, and a 543-kbp pAt58 plasmid (GenBank accession numbers AE007869, AE007870, AE007871, and AE007872, respectively).

Some strains contain additional plasmids, such as pTar, which carries genes necessary for the use of tartrate, abundant in grapevine, in *A. vitis* S4. Sequencing data also confirmed the relatedness to *Rhizobium* species, especially *R. etli*, *R. leguminosarum*, and *Sinorhizobium meliloti*. Specifically, the circular chromosomes (but not other genetic elements) show extensive nucleotide colinearity and gene order conservation between these species, supporting the view that *Agrobacterium* and *Rhizobium* share recent common ancestors and diverge

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Table 1 Genome structure of representative strains of *Agrobacterium* biovars

Representative strain	*Biovar*	*Genome composition*
Agrobacterium tumefaciens C58	I	Circular chromosome Secondary linear chromosome Two plasmids
Agrobacterium radiobacter K84	II	Circular chromosome Four plasmids
Agrobacterium vitis S4	III	Circular chromosome Secondary circular chromosome Five plasmids

following the acquisition of plasmids that confer pathogenicity or symbiosis, respectively.

Agrobacterium as Phytopathogen

Horizontal Gene Transfer

Strains harboring a Ti/Ri plasmid have the remarkable – and, in fact, unique in the living nature – capacity to transfer a single-stranded copy, termed the 'T-strand', of the T-DNA segment of the Ti plasmid into the host cell, which may be followed by its stable integration in the cell genome. This DNA transfer from *Agrobacterium* to host plant represents the single known example of horizontal gene transfer naturally occurring between a bacterium and a eukaryotic organism. Intriguingly, the capability to genetically transform its hosts – for decades considered unique to *Agrobacterium* – may also occur with other pathogenic bacteria (at least under experimental conditions), such as zoonotic pathogen *Bartonella henselae*.

A set of genes encoded by the Ti plasmid and termed the 'virulence (*vir*)' genes – which comprise seven major loci (*virA*, *virB*, *virC*, *virD*, *virE*, *virG*, and *virH*) and encode most components of the protein machinery of genetic transformation – is required to mediate the mobilization of the T-strand from the Ti-plasmid, its export to the host cell cytoplasm, import into the host cell nucleus, and integration into the host genome.

Expression of the *vir* genes is induced by small molecules secreted by wounded plant tissues, such as phenolics (e.g., acetosyringone) and mono- and di-saccharides. Furthermore, in addition to the pTi-encoded *vir* genes, several chromosomal loci are involved in the infection process, facilitating bacterial attachment to the host cell and enhancing the efficiency of the T-DNA transfer.

Transferred Genes and Infection Symptoms

In nature, a successful T-DNA transfer results in proliferation of the transformed plant cells, that is, cells harboring one or more T-DNA copies within their genome. Depending on the identity of the genes encoded by the T-DNA, the visible symptoms of this genetic transformation event include neoplastic growths, such as leaf or crown galls, or hairy roots. Generally, the protein products of the T-DNA genes fall into two categories: factors involved in host cell neoplasia and enzymes necessary for the synthesis and export of opines. Whereas the function of some of these genes is well known, many others remain functionally uncharacterized. The known products of the T-DNA genes causing neoplasia, that is, oncogenes, include enzymes involved in metabolism of and response to plant hormones, such as auxins and cytokinins that regulate cell growth, as well as proteins that regulate cell division via chromatin remodeling. Plant cells transformed with and expressing these genes can thus proliferate without exogenous supply of growth regulators.

While the oncogenes create a mass of proliferating host cells, the opine biosynthesis and export genes direct these cells to supply the bacteria with nutrients. Opines represent a group of small molecules derived from the condensation of an amino acid and a keto-acid or a sugar, which *Agrobacterium* uses as a source of carbon and nitrogen. There are at least 30 different opines, and each *Agrobacterium* strain carries genes required for the synthesis of a specific set of opines in the transformed plant tissues and for catabolism of these opines after they have been secreted from the host neoplasm.

Host Range

In nature, *Agrobacterium*-induced tumors have been documented on more than 1000 different plant species, belonging to most of the families of the dicotyledonous plants. By contrast, only a small fraction of monocotyledonous species, for example, *Liliaceae*, are susceptible to the *Agrobacterium* infection, whereas most monocots, which include such agronomically important crop species as cereal grains (e.g., the *Gramineae* family), are recalcitrant to transformation. Under laboratory conditions, the range of eukaryotic species that can be genetically transformed by *Agrobacterium* is exceedingly broad, and it includes almost all plant species, yeast and many other fungal species, and even cultured human cells. This promiscuity of *Agrobacterium* indicates the conserved nature of the transformation process.

Agrobacterium as Genetic Engineering Tool

The discovery that crown gall formation is due to the transfer and stable integration of a bacterial DNA into the plant genome directly led to the exploitation of this capability for genetic manipulation of plants to introduce into their genomes, at will, genes that confer new and desirable traits. This exploitation is based on the property of the T-DNA which is defined by its short, 25-bp borders such that any DNA sequence placed between them can function as T-DNA and, thus, be transferred to the host cell. Methodologies were developed to replace the native T-DNA genes with one or more genes of interest to be expressed in plants. *Agrobacterium* harboring such an engineered Ti plasmid is allowed to infect the target plant, and the transformed tissues are then regenerated into a healthy and fertile plant stably expressing the newly introduced genes.

The ability to generate transgenic, or genetically modified (GM), plants represented a revolutionary development for the entire fields of plant biology and plant biotechnology. It enabled studies of plant growth and development, plant physiology, plant ecology, and plant pathology on a molecular level, for example, by expressing a gene of interest in any plant tissue and throughout the entire plant life cycle.

Furthermore, because T-DNA insertion is essentially random in the host genome, it was also used to generate extensive collections of T-DNA insertion mutants, particularly for the model plant *Arabidopsis thaliana*; because each such mutant contains T-DNA sequences within the mutation site, the mutated gene can be easily identified and mapped to the mutant phenotype. The importance of this approach was so overwhelming that studies were initiated to extend the mutagenesis technology to species that are not usually infected by *Agrobacterium* in nature, such as monocot plants and even fungi.

Conversion of the Ti plasmid into a custom-made genetic engineering vector also allowed production of crops with agronomically important traits. So far, the main commercially successful applications resulted from plants engineered for resistance to insect pests via transgenic expression of a bacterial insecticidal protein from *Bacillus thuringiensis*, or for tolerance to novel, ecologically more acceptable herbicides. These traits were genetically introduced into the élite lines of major crops, such as corn, soybean, canola (rapeseed), potato, tomato, and cotton. In 2009, about 134 million hectares (330 million acres) of different transgenic crops were grown, mostly in North and South America and China. More recent applications involve engineering of crop plants better adapted to biotic and abiotic stresses as well as generation of transgenic plants that produce large quantities of pharmaceuticals, a strategy known as 'biopharming'. Besides *A. tumefaciens*, biopharming also utilizes *A. rhizogenes* to produce pharmaceuticals in hairy root cultures. Finally, *Agrobacterium* is increasingly used for genetic transformation of many species of fungi, where it often allows better transformation efficiency and stability of the transgenes as compared to more conventional fungal transformation methods.

Agrobacterium as Experimental System for Fundamental Cell Biology

In the last three decades, *Agrobacterium* interactions with its host cells have emerged as a powerful experimental system for studies of fundamental aspects of general cell and molecular biology. For example, the study of the *Agrobacterium* type 4 secretion system (T4SS) has provided the molecular model for this secretion apparatus, widespread in the bacterial world. Unraveling the function of *Agrobacterium* effectors transported in the host cell has also been essential for probing many basic mechanisms in host plant cell, such as DNA and protein trafficking inside eukaryotic cell, nuclear targeting of proteins and protein–nucleic acid complexes, or involvement of the ubiquitin/proteasome (UPS) system in bacterial infection. In addition, the functional study of T-DNA gene products led to significant advances in our understanding of regulation of plant growth and cell division.

See also: Agrobacterium and Ti Plasmids; Transfer of Genetic Information from *Agrobacterium tumefaciens* to Plants.

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