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Applications of *Agrobacterium tumefaciens* in the biotechnology

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Abstract

Agrobacterium tumefaciens is a ubiquitous bacterium known to cause plant diseases, particularly crown gall or tumor disease. This pathogen can drastically reduce crop yields, impacting agricultural productivity and having economic implications for producers. Diseases caused by *Agrobacterium tumefaciens* can disrupt local ecosystems, and its presence in infected plants creates an imbalance in flora and fauna. These diseases not only affect crops of interest but can also impact other species within the ecosystem. Managing this infection is essential to maintaining crop and ecosystem health; therefore, continuous monitoring and the promotion of management strategies to mitigate the pathogen's effects are necessary. However, *Agrobacterium tumefaciens* has been used as a biotechnological tool due to its ease in transferring genetic material between species, and given this property, it can introduce genes of interest that may provide an application for improving a crop, such as resistance to pests or promoting the application of gene therapy in humans or the overproduction of some metabolite of interest with beneficial agronomic and human properties.

Keywords: *Agrobacterium Tumefaciens*; Galls; Crown; Pathogen; Plants; Application; Biotechnology

1. Introduction

Agrobacterium tumefaciens is a ubiquitous phytopathogenic bacterium known to cause plant diseases, particularly crown gall or tumor disease. It has also been implicated in opportunistic infections in humans, such as endocarditis, peritonitis, urinary tract infections, and pneumonia, with the species *A. radiobacterium* and *A. tumefaciens* being implicated [1][2]. As a phytopathogen, *Agrobacterium tumefaciens* can drastically reduce crop yields, impacting agricultural productivity and having economic implications for producers. Diseases caused by *Agrobacterium tumefaciens* can disrupt local ecosystems, and its presence in infected plants creates an imbalance in flora and fauna. These diseases not only affect crops of interest, such as some monocots and primarily dicots, but can also affect other species within the ecosystem. *Agrobacterium* migrates from the host cell to the cytoplasm and subsequently to the nucleus [3]. Managing this infection is essential for maintaining crop and ecosystem health, requiring continuous monitoring and the implementation of management strategies to mitigate the pathogen's effects. Effective control of diseases caused by *Agrobacterium tumefaciens* necessitates the implementation of various strategies. Integrated pest management involves monitoring and establishing the presence of *Agrobacterium tumefaciens* in the environment, employing cultural practices such as crop rotation, and using plant varieties resistant to the pathogen. Furthermore, this bacterium's ability to transfer genetic material between species of different kingdoms, such as prokaryotes and plants, has been used to explain the appearance of tumors. This characteristic is used as a tool for biotechnological studies involving the introduction of genetic material into cells (transformation). In recent years, the genetic transformation of fungal and animal cells has been demonstrated [4]. These practices not only reduce the pathogen population but also minimize the need for pesticides. The use of biological control methods promises to manage *Agrobacterium*. Certain beneficial microorganisms, including some bacteria and fungi, can inhibit *Agrobacterium* growth

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or limit its ability to infect plants. The use of these biological control agents can help maintain ecological balance and provide a sustainable alternative to chemical pesticides. Plants transformed using *Agrobacterium tumefaciens* have been engineered to express a specific trait, such as herbicide resistance, production of a new compound, or improved productivity [5]. Breeding plants for resistance is another strategy. By selecting and developing varieties that exhibit natural resistance to *Agrobacterium*, growers can now produce crops that are less susceptible to infection. Advances in genetics and genomics have accelerated this process by enabling the identification of resistance genes. However, it is essential to maintain resistant varieties and prevent the emergence of new pathogenic strains. Biotechnology plays a crucial role in mitigating the impact of *Agrobacterium* on crops. Techniques such as gene editing and transgene introduction can enhance plant resistance to bacterial infections by integrating T-DNA into the cell's genome. Although VirD2 functions as a ligase, other enzymes, such as polymerases, may be involved. These polymerases convert the single strand of integrated T-DNA into a double strand during the cell transformation process in plants [4]. Furthermore, biotechnological methods can be used to develop diagnostic tools for the early detection of *Agrobacterium*, facilitating timely intervention and control.

2. Results and discussion

2.1. Some applications of transformations using *Agrobacterium tumefaciens*

For decades, *Agrobacterium tumefaciens* has been used as a biotechnological tool for developing methodologies for constructing transgenic plants, primarily dicotyledonous plants and, to a lesser extent, monocotyledonous plants. Table 1 presents some examples of transgenic plants and their characteristics obtained using *Agrobacterium tumefaciens* as a biotechnological tool. Plants transformed using *Agrobacterium tumefaciens* express certain characteristics such as herbicide resistance, production of new compounds, and increased productivity. For example, Lee *et al*. reported the genetic transformation of canola (*Brassica napus*) with a gene encoding an oleosin protein cloned from maize, achieving gene expression in some tissues. Because oleosins are important proteins for their role in lipid production and storage in seeds, increasing the expression of these proteins is essential for improving nutritional characteristics and lipid production in oilseed crops [5]. Jeknic *et al*. reported a genetic transformation in *Iris germanica*, a monocotyledonous ornamental plant that produces ketone compounds with a violet aroma, important in the cosmetics industry. However, this plant presents some problems such as interspecific incompatibility, so transformation work opens up other possibilities for genetic improvement, using *Agrobacterium tumefaciens*, and the economic impact of the crops [6].

Table 1 Some examples of the use of *Agrobacterium tumefaciens* as a biotechnological tool.

Protocol used	**example**	**reference**
Tissue infection using *Agrobacterium tumefaciens* strain A2008	Tobacco (*Nicotiana tabacum*) resistant to insects due to the production of the *Bacillus thuringiensis* toxin in its tissues	Perlak and collaborators, 1990 [7].
Transformation of hypocotyls by infection with *Agrobacterium tumefaciens*	Overproduction of α and β-carotene in canola seeds (*Brassica napus*)	Shewmaker *et al*., 1999 [8].
Coculture of embryos with *Agrobacterium tumefaciens* strain LBA4404	Development of golden rice, a variety of *Oryza sativa* that produces and accumulates β-carotene in the grains, as a precursor of vitamin A.	Ye *et al*., 2000 [9].
Infection of leaf discs with *Agrobacterium tumefaciens* strain EHA105.	Production and accumulation of astaxanthin, a carotenoid of bacterial origin in tobacco (*Nicotiana tabacum*)	Mann *et al*. 2000 [10].

2.2. Engineering to Transform Target Cells into Plants

During pathogenesis, *Agrobacterium tumefaciens* encounters a variety of factors that limit transformation efficiency. Plant regeneration after T-DNA integration is another significant obstacle for many plant species and genotypes. Factors that modulate *Agrobacterium tumefaciens*-mediated transformation exist, and recent genetic engineering strategies increase plant regeneration efficiency.

The induction and fixation of virulence by vir genes, which interact with small molecules in the host tissue, induces *Agrobacterium tumefaciens* virulence. Additionally, the presence of calcium ions in the soil influences bacterial polar attachment to root hairs [11]. Low concentrations of calcium added to *Agrobacterium tumefaciens* suspension positively regulate polysaccharide production and polar attachment, as well as increasing transformation efficiency in callus propagation [12]. It is important to note that the calcium ion also modulates the architecture of the plant cell wall, in the interactions between homogalacturonan pectic polysaccharides, which increases cell wall rigidity [13]. It is well known that the cell wall is a natural barrier against pathogen attack, and the loosening of the cell wall of both the host plant and *Agrobacterium tumefaciens* is important for transformation. Therefore, several studies have been conducted to identify the genetic basis of plant resistance to *Agrobacterium tumefaciens*. Genetic screening studies have been conducted on mutant strains of *A. thaliana* resistant to transformation by *Agrobacterium tumefaciens* (rat a), identifying several cell wall synthesis genes, including a mannose synthase (rat 4), β-expansin (rat 18), and an arabinogalactan (AGP) protein or extensin-like glycoproteins (rat 1 and rat 3) [14][15]. These enzymes play an important role in cell wall loosening and induce the release of sugars synergistically with vir genes associated with plant-derived phenolic compounds. Furthermore, the high-lysine arabinogalactan 17 protein (AGP17/rat 1) was found to be essential for the polar attachment of *Agrobacterium tumefaciens* to plant tissue, among other functions [16].

3. Conclusions

Agrobacterium tumefaciens is a plant pathogenic bacterium that can infect some crops, affecting their production. However, it possesses some interesting characteristics that can be leveraged for applications in agricultural biotechnology, such as diagnostic development. Understanding the plant-microorganism interactions is crucial, as the regulation of virulence induction and fixation is essential for efficient transformation into *Agrobacterium tumefaciens* and the host. Early detection of the most aggressive pathogens is paramount for transforming plants and making them more resistant to these opportunistic pathogens. This facilitates intervention through gene expression or silencing and/or the development of gene therapy for a rapid response in phytopathogen control, among other applications.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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