Chapter

# Application of Radiation and Genetic Engineering Techniques to Improve Biocontrol Agent Performance: A Short Review

*Seyed Mahyar Mirmajlessi, Hossein Ahari Mostafavi, Evelin Loit, Neda Najdabbasi and Marika Mänd*

## Abstract

Biological control is a potential nonchemical method to manage plant pathogens by beneficial microorganisms. To improve antagonistic potential of biocontrol agents, mutation by radiations, chemicals, and genetic manipulations has been used. Genetic techniques and ionizing radiation containing direct or indirect emissions play the greatest role for selection of useful microorganisms to enhance the efficiency of biological systems. Indeed, genetic engineering has a main role in increasing antimicrobial metabolites, host colonization ability, and endurance in micro-ecosystem. Genetic improvement can be achieved by protoplast fusion, genetic modification (GM), and chemical (genotoxic agents) and physical mutations. However, ultraviolet light and ionizing radiations can induce modifications in the genome of an organism. Irradiation, particularly gamma rays, is also applied for controlling postharvest diseases. Indeed, irradiation cannot completely eliminate pathogens, but it might result in cell injury and directly damage the chromosomal DNA of a living cell. This technology has been used for many reasons including disinfestation of foods, reducing foodborne pathogens, and extending shelf life many fruits, vegetables, and nuts. In the current review, we discuss advances in the radiation and molecular genetic techniques with the aim to improve antagonistic potential of microorganisms as it is applied to the suppression of plant pathogens.

**Keywords:** biocontrol agents, genetic engineering, ionizing radiations

## 1. Introduction

The risks associated with chemical residues on the leaves and fruits have highlighted the need for more useful and safer alternative control treatments. Biological control is a potential nonchemical mean to manage plant pathogens including fungi, bacteria, nematodes, or weeds by beneficial microorganisms such as fungi, yeast, or bacteria [1]. The virulence of pathogens probably changes if genetic mutations occur in the genes related to pathogenicity of microorganisms upon mutagenic treatments [2]. It is quite obvious that the application of ionizing radiations such as gamma rays and X-rays and genetic methods plays the greatest role in the development of techniques for the selection of useful microorganisms

[3]. Indeed, irradiation is the process of exposing an amount of energy in the form of speed particles or rays for improving food safety and reducing microorganisms that destroy agricultural crops. For instance, studies on transcriptional changes of *Salmonella typhimurium* using gamma irradiation showed that the expression of the virulence genes in irradiated mutants was reduced in comparison with non-irradiated controls [4]. It has been also shown that gamma irradiation alone or in combination with other methods can improve the microbiological safety [5]. Likewise, the wholesomeness (lack of teratogenicity, mutagenicity, and toxicity) of irradiated products has been studied extensively, in which food irradiation has not provided any evidence of increased threat of mycotoxin formation in irradiated food [6]. There is an increasing interest in commercial application of genetically modified microorganisms with improved biocontrol properties toward plant pathogens [7]. To that end, this chapter is an advanced survey reviewing the radiation and molecular genetic techniques with the aim to improve antagonistic potential of microorganisms as it is applied to the suppression of plant diseases.

## 2. Genetic improvement of microorganisms

The biological and molecular characterizations of biocontrol agents and bioactive compound producers are very important for the modern agriculture [8]. Since environmental conditions are subject to change, the biocontrol agent requires genetic improvement for effective performance [9]. To improve the efficiency and productivity of biological systems, genetic engineering has a main role in increasing antifungal and antibacterial metabolites, host colonization ability, and endurance in micro-ecosystem. Genetic improvement can be achieved by chemical and physical mutations, protoplast fusion, and transformation [10].

### 2.1 Use of protoplast fusion

Protoplast fusion is an important technique for gene manipulation. It breaks down the barriers to genetic exchange and is a relatively new flexible technique to induce or promote genetic recombination in a variety of prokaryotic and eukaryotic cells [11]. By protoplast fusion (**Figure 1**), interspecific or even intergeneric hybrids can be produced, and it is feasible to transfer useful genes, for attributes (such as disease resistance, enzyme and phytotoxin production, rapid growth rate, nitrogen fixation, protein quality, and drought resistance), from one species to another [12].

To breed new strains with improved production of the spore and phytotoxin, protoplast fusion between the *Helminthosporium gramineum* subsp. *echinochloa* and *Curvularia lunata* was carried out [10]. Prabhavathi et al. [13] showed that the isolated protoplast from *Trichoderma reesei* strain PTr2 significantly increased enzyme activities in two fusants SFTr2 and SFTr3 as compared to the parental strain PTr2. Bakhtiari et al. [14] produced a new recombinant of *Tolypocladium inflatum* with cyclosporine 2.8 times more than parental strain by protoplast fusion between different strains.

### 2.2 Genetic transformation

Nowadays, some wild-type fungal and bacterial species are used as biocontrol agents. But, there are limitations that restrict their efficacies. Poor survival of the inoculant in particular soils and low production of required metabolites are the most important limitations [15]. The technology of genetic modification (GM) has the capacity to create new strains in which these problems are overcome [16]. Genetic manipulation requires the development of vector-mediated transformation

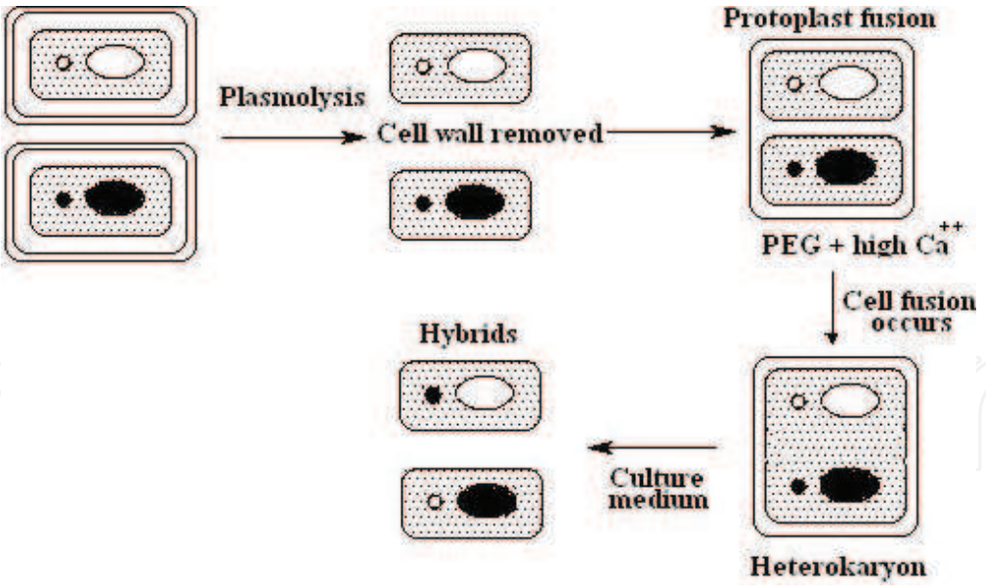


**Figure 1.**
*Schematic illustration of haploid cell construction by protoplast fusion.*

systems that include, first, infusion of exogenous DNA into receiver cells; second, expression of genes present on the incoming DNA; and, finally, stable preservation and replication of the inserted DNA, leading to the expression of the desired phenotypic trait (**Figure 2**).

The development of an effective strain that can tolerate the environmental adversities and securing of the essential regulatory approval are primary obligations for the successful use of a GM inoculant as biocontrol agents [17]. Resca et al. [18] genetically modified the strains of *P. fluorescens* F113Rif (pCU8.3) and *P. fluorescens* F113Rif (pCUP9) to increase phenazine1 carboxylate (Phl) production for biocontrol efficacy against *Polymyxa betae* on sugar beet. An endochitinase-encoding gene was cloned from the *T. viride*. This gene was introduced into *Chaetomium globosum* CG10 after ligating it with the promoter and terminator of Trp synthetase from *Aspergillus nidulans*. Endochitinase activity significantly increased in 30% of the transformants for improvement of the biocontrol activity of *Chaetomium globosum* [19].

## 2.3 Improvement of the bioagents via mutagenesis

Breeding of antagonists is directed to achieve effective strains for biocontrol of plant pathogens under a wide range of environmental conditions. Mutation techniques have been used to improve antagonistic potential of biocontrol agents to manage phytopathogens. Physical and chemical mutagens have been applied by many researchers to generate new biotypes [3]. Ultraviolet light (UV), ionizing radiation, and chemicals (as genotoxic agents) can randomly induce modifications in organism's genome. About 25 years ago, X-ray, as an active mutagen, was used to produce the first mutant strain of *Penicillium chrysogenum*. The next mutagenic technique used was the application of ultraviolet light on *P. chrysogenum*. The produced mutant displayed three times more activity (with respect to penicillin contains) than the original strain induced by X-irradiation.

Later on, fast neutrons and gamma irradiation were used successfully. The effectiveness of gamma rays significantly surpassed that of UV and X-rays. Also, mutagenic effects of chemicals in combination with UV light on *P. chrysogenum* were investigated. The mutant strains were about 1500 times as productive as the wild type [19]. Mess et al. [20] demonstrated that gamma irradiation at 130 Gy using 137Cs generated an avirulent mutant (avr-mutant) of *F. oxysporum*

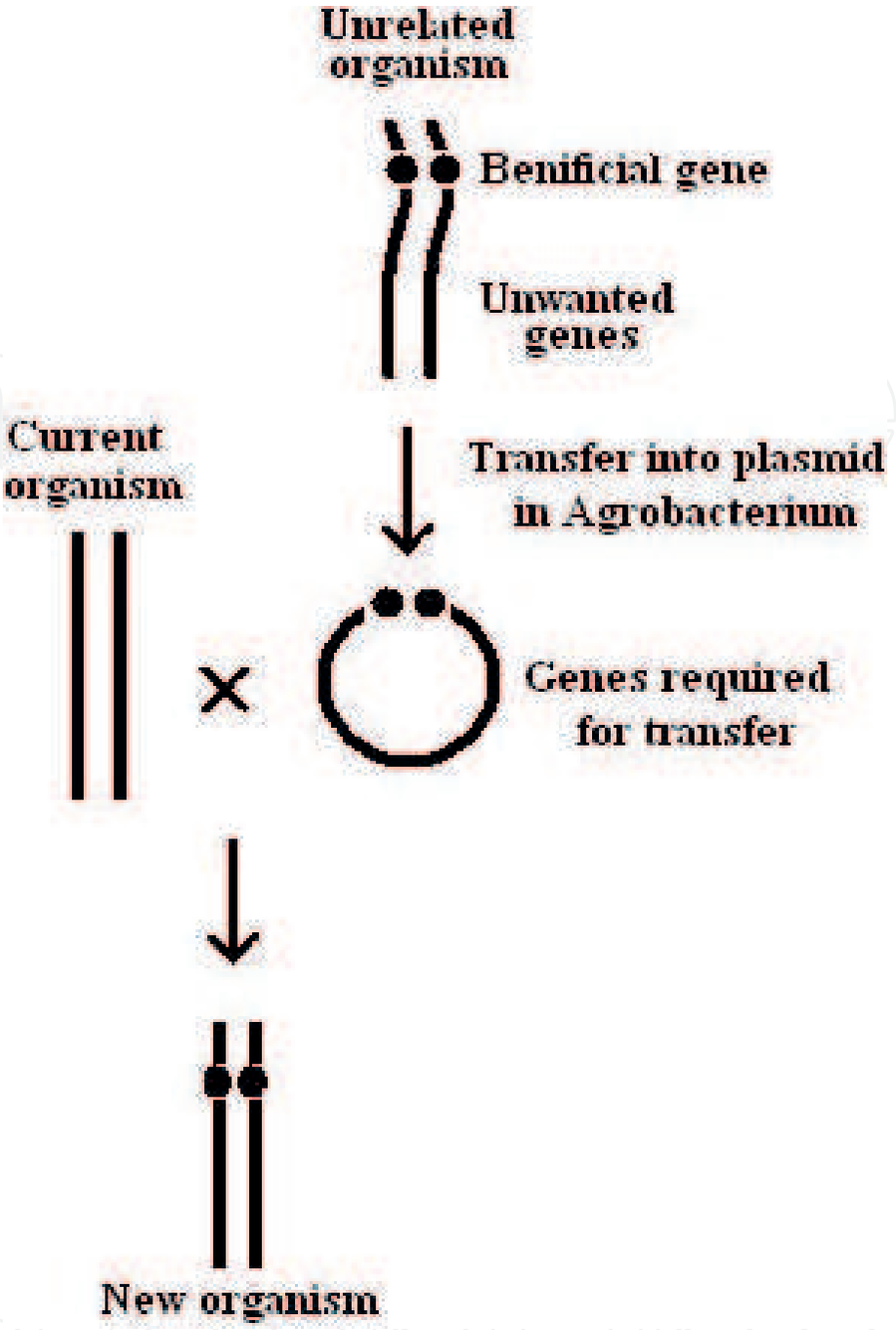


**Figure 2.**
*Schematic illustration of plasmid-mediated transformation system.*

f. sp. *lycopersici*. One mutant showed the expected loss of fusarium root rot on tomato plants. Some successful studies have been made to improve the biocontrol potential of *Trichoderma* and *Gliocladium* species by exposing them to physical mutagens such as gamma ray. Mutagenesis of *Trichoderma atroviride* by gamma irradiation greatly improved their capabilities to produce antibiotics [21]. Rugthaworn et al. [22] investigated the improvement of Actinomycete strains by gamma irradiation for biocontrol of *Fusarium sporotrichioides*, *Rhizoctonia solani*, and *Sclerotium rolfsii*. The induced mutants were detected by observing the ability of chitinase production and showed higher inhibitory effect on three phytopathogenic fungi than the wild type. Mohamed and Haggag [23] showed that gamma irradiation generated a *T. harzianum* mutant against *F. oxysporum* through increasing of antifungal metabolites (hydrolytic enzymes, antibiotics, and total phenols) under salt stress conditions.

Irradiation of *T. harzianum* produced salt-tolerant mutants that greatly were improved in sporulation, growth rate, and biocontrol ability against *Fusarium oxysporum*, the causal agent of tomato wilt disease. Ahari-mostafavi et al. [24] showed the possibility of biological control of bean root rot disease, using of avirulent mutants of *Fusarium solani* f. sp. *phaseoli* isolate produced by gamma irradiation. Furthermore, stable gamma-irradiated mutants of *G. virens* have immense potentiality as biocontrol agents [25]. Haggag [26] improved the production of *P. fluorescens* antibiotics (including phenazine, pyrrolnitrin, and phloroglucinol) and siderophore against damping-off pathogens (*F. solani*, *F. oxysporum*, and *R. solani*) by UV light. Papavizas et al. [27] developed mutant isolates of *G. virens* by means of irradiation and a chemical mutagen. They showed evidence that it is feasible to induce benomyl-tolerant mutants of *G. virens* by long and repeated exposure to UV irradiation combined with ethyl methanesulfonate. Mohamed et al. [7] applied the UV mutagenesis technique to enhance three hydrolytic enzymes effective in the biocontrol ability of *T. viride* against two of the important plant fungal pathogens, *Sclerotia rolfesii* and *Sclerotinia sclerotiorum*. In biological control experiments against root rot and white rot diseases caused by *S. rolfesii* and *S. sclerotiorum*, respectively, in bean plants under artificial and natural infested soil, complete control of the disease was achieved. Treatment of the bean seeds with *T. viride* mutants resulted in reducing colonization of *S. rolfesii* and *S. sclerotiorum* in bean rhizosphere compared with treatment with their parental wild type and increased plant yield.

Moreover, Balasubramanian et al. [28] tested biocontrol efficacy of the UV mutants and wild strain of *T. harzianum* against phytopathogens such as *F. oxysporum*, *Bipolaris oryzae*, *R. solani*, and *Alternaria* sp. A mutant strain showed maximum inhibition of the above pathogens through more extracellular chitinase and protein production, when compared to the wild strain. Also, UV mutagenesis increased 2.29-fold in phytase activity of *T. lanuginosus* over that of their parental strain [29]. Chitinase production was improved by sixfold in *Ophiostoma floccosum* using UV mutagenesis [30]. Furthermore, the ethyl methanesulfonate (EMS) mutant with an enzyme activity of 25.56 U $ml^{-1}$ was obtained by further exposure to UV radiation and yielded an activity of 34.12 U $ml^{-1}$ [31].

### *2.3.1 Mechanism of ionizing radiation effects on bioagents*

Irradiation can have direct or indirect effects on organisms. In the case of a direct hit, electromagnetic radiance and high-energy particles break chemical bonds or reactive oxygen-centered (•OH) radicals originated from the radiolysis of water. Hydroxyl radicals are very reactive and are known to interfere with the bonds between nucleic acids within a single strand or between opposite strands. An indirect effect occurs on organisms when radiation ionizes a neighboring molecule, which in turn reacts with the genetic material [32]. Ionizing radiation effects (on microorganisms) are primarily the result of disruption of the DNA or RNA (**Figure 3**).

Since the DNA is much larger than other molecular structures in a cell, the induced biological and chemical changes by either primary ionizing or through secondary free radical attack can prevent replication and destroy cells [33] or generate some genetic traits of microorganisms to have higher antagonistic potential [2]. Therefore, the induced biological and chemical changes are related to the absorbed radiation dose [33]. The pathogenicity of infectious organisms is diminished by irradiation. Lim et al. [4] showed that the expression of the virulence genes in *S. typhimurium* gamma-irradiated mutants was reduced and expression of toxin genes of *Vibrio* spp.-irradiated mutants did not increase, compared with non-irradiated wild types.

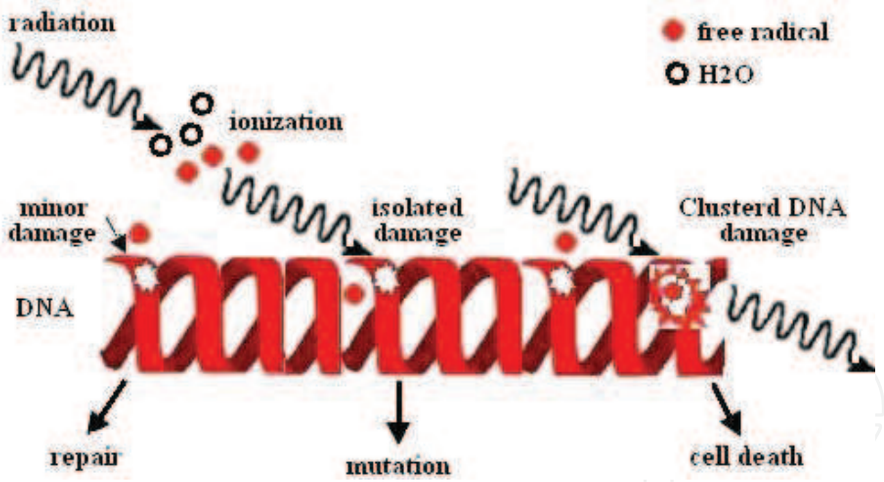


**Figure 3.**
*Schematic illustration of DNA mutation by ionizing mutations drawn by anonymous.*

### *2.3.2 Irradiation sources*

Ionizing radiation is mainly known to high-energy photons of radio nuclides (gamma rays) and X-rays from machine sources with energies up to 5 MeV and accelerated electrons with energies up to 10 MeV generated by electron accelerating machines [34]. Gamma ray occurs in the short wavelength, with high-energy region of the spectrum, and has the greatest penetrating power. Gamma rays come from spontaneous breakdown of radionuclides including the radionuclide's cobalt-60 (60Co) with a half-life of 5.3 years or cesium-137 (137Cs) with a half-life of 30 years [35]. X-ray is similar to gamma radiation based on radioactive isotopic sources. Although their effects on materials are generally similar, these kinds of radiations differ in their energy spectra, angular distributions, and absorbed dose rates [36, 37]. Electrons can be produced from machines capable of accelerating electrons as light speed by means of a linear accelerator. In comparison with gamma ray and X-rays, electrons cannot penetrate very far into materials and do not have deep penetrating power. So, electrons as beta particles are usually chosen to treat the surface of materials [32, 34]. Generally, the strength of the source and the length of time a material is exposed to the ionizing energy determine the irradiation dose, measured in grays (Gy) or kilo grays (1 kGy = 1000 Gy). One gray is equal with one joule of energy absorbed in a mass of one kilogram [38]. Based on available evidence, the safety of the irradiation technology in food industry was considered and judged acceptable. This has developed in international bodies such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA), the and Codex Alimentarius Commission [35, 39].

## 3. Conclusion

Plant pathogens are a worldwide problem. A variety of approaches may be applied to control plant diseases. Beyond suitable agronomic performances, farmers frequently rely on chemicals. Environmental pollution and carcinogenic effects of pesticides are limiting factors in the success of their application. Nowadays, there are severe regulations and political pressure to remove the most dangerous chemicals from the market. So, biological control could be the best alternative against plant pathogens, and development of mutants is an important technique in strain

improvement toward plant pathogen suppression, which yields reliable strains for biocontrol. Since strains bred by mutagenesis can get registration (from environmental protection agencies) more easily than strains produced by protoplast fusion and transformation or via gene cloning for field use, more attention should be paid to the mutagenic methods. In an agricultural environment, mutants are interacting and competing with various communities of microorganisms that can have intense effects on the survival and performance of the introduced mutants. Hence, before commercial application of such inoculants in an open environment, their behaviors and potential impact on ecosystems should be investigated as part of the risk assessment. Additionally, integrated treatment of irradiation and biocontrol agent has the potential as an alternative means for postharvest disease control. In fact, there is a limited dose rate for its application on postharvest diseases of fruits and vegetables. Thus, the combination of irradiation and biocontrol agent increases applied range of irradiation for postharvest control by decreasing of dose rate to which the product has been exposed. From our point of view, considering different aspects of irradiation may provide useful information for managing harmful microorganisms on crops, so that in the near future, this technique will be used as one of the most important research tools for biotechnologists, plant pathologists, and molecular biologists.

## Acknowledgements

Authors want to thank Prof. Vahe Minassian from Tarbiat Modares University for critical reading of the manuscript.

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