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Significant Achievements and Present Status of *Trichoderma spp*. in Bio control of Plant Diseases

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SUMMARY

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. The word *Trichoderma* is taken from thrix = hair and derma = skin. The genus *Trichoderma* was first proposed as a genus more than two hundred years ago by Persoon in 1794 at Germany. There are 89 species in the *Trichoderma* genus. *Hypocrea* are teleomorphs of *Trichoderma* which themselves have *Hypocrea* as anamorphs. In recent years, genetic improvement of *Trichoderma* by induction of mutation using mutagens has successfully been attempted for improvement of potential ability of biocontrol agent. Mutation in *Trichoderma* results in beneficial and harmful effect on morphological, physiological, biochemical and molecular properties. Induced mutation is one of the most commonly used practices to modify the genetic composition of antagonistic fungi. Mutation on *Trichoderma* sp. by UV and gamma radiation brought about changes in morphological features like colony diameter, sporulation, dry mycelial weight and enzymes like ß-1,3-glucanase, ß-1,4-glucanase, cellulase and antibiotics like trichodermine, gliotoxin and virindin. There was a potential increase in antifungal metabolites of the selected mutants as compared to wild parents. Mutants of *Trichoderma* sp. were found to be a better biocontrol agent against phytopathogens. Selection of beneficial mutant of biocontrol agent becomes a better option for management of plant pathogens.

INTRODUCTION

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. *Trichoderma* is free living, asexually reproducing and filamentous fungi. Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from soil, decaying wood and other forms of plant organic matter. The genus *Trichoderma* was first proposed as a genus more than two hundred years ago by Persoon in 1794 at Germany. *Trichoderma*, a fungi, which grow saprophytically in soils have proved as an effective biocontrol agent of soil borne plant diseases specifically wilt caused by different pathogens like; *Pythium* spp., *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Phytophthora* spp. They grow trophically towards hyphae of other pathogenic fungi, coil them and degrade their cell walls. This process is called "mycoparasitism", which limits the growth and activity of plant pathogenic fungi. *Trichoderma* species are strongly antagonistic to other fungi. Appears to kill other fungi with a toxin and lytic enzymes. Can be serious pests in cultivated mushroom beds. Many species of *Trichoderma* have been studied, several developed into products. The important species exploited are *Trichoderma viride* and *Trichoderma harzianum. T. hamatum, T. Viride* and *T. Harzianum* common species used in biological control, it is an avirulent plant symbiont *Trichoderma* that occurs in all agricultural soils. Highly competitive and displays antagonism against other pathogenic fungi. Successfully

cultured for use as a biofungicide. In recent years, genetic improvement of *Trichoderma* by induction of mutation using mutagens has successfully been attempted for improvement of potential ability of biocontrol agent. Mutation in *Trichoderma* results in beneficial and harmful effect on morphological, physiological, biochemical and molecular properties. The most important *Trichoderma* species in industrial, medical and biocontrol uses include:

- Trichoderma hamatum
- *Trichoderma harzianum*
- *Trichoderma koningii*
- *Trichoderma longibrachiatum*
- *Trichoderma reesei*
- Trichoderma virens
- *Trichoderma viride*

Characteristics

- Tolerant to cool and warm temperatures.
- Tolerant to low moisture.
- Tolerant to many fungicides.
- Prefers acidic soils (pH 3.5 to 4.5).
- Rhizosphere competent.

Mutation

In genetics, a mutation is a change in the nucleotide sequence of the genome of an organism, virus, or extrachromosomal genetic element. Mutations result from unrepaired damage to DNA or to RNA genomes (typically caused by radiation or chemical mutagens), from errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements(Mutations may or may not produce discernable changes in the observable characteristics (phenotype) of an organism. Mutations play a part in both normal and abnormal biological processes, including evolution, cancer, and the development of the immune system. Mutation can result in several different types of change in sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. One study on genetic variations between different species of *Drosophila* suggests that if a mutation changes a protein produced by a gene, the result is likely to be harmful, with an estimated 70 percent of amino acid polymorphisms having damaging effects, and the remainder being either neutral or weakly beneficial. Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as DNA repair to prevent mutations.

Illustration of three types of point mutations.

- A point mutation, or single base substitution, is a type of mutation that causes the replacement of a single base nucleotide with another nucleotide of the genetic material, DNA or RNA. The term *point mutation* also includes insertions or deletions of a single base pair.
- A point mutant is an individual that is affected by a point mutation.
- Repeat induced point mutations are recurring point mutations, discussed below.

Causes of point mutations

Point mutation is a random mutation in the deoxyribonucleic acid (DNA) that occurs at one point. This mutation can be a deletion, transition, insertion, or transversion. Point mutations usually take place during DNA replication. DNA replication occurs when one double-stranded DNA molecule creates two single strands of DNA that is a template for the

creation of the coinciding strand. A single point mutation can change the whole DNA sequence. Changing one purine or pyrimidine may change the amino acid that the nucleotides code for.Point mutations may arise from spontaneous mutations that occur during DNA replication. The rate of mutation may be increased by mutagens. Mutagens can be physical, such as radiation from UV rays, X-rays or extreme heat, or chemical (molecules that misplace base pairs or disrupt the helical shape of DNA). Mutagens associated with cancers are often studied to learn about cancer and its prevention.

There are multiple ways for point mutations to occur. First, ultraviolet(UV) light and higher-frequency light are capable ionizing electrons and in turn impacting DNA. Also, one of the cell metabolic byproducts, reactive oxygen molecules with free radicals, can also be very harmful to DNA. These reactants can lead to both single-stranded DNA breaks and double-stranded DNA breaks. Third, bonds in DNA eventually degrade, which creates another problem to keep the integrity of DNA to a high standard. There can also be replication errors that lead to substitution, insertion, or deletion mutations.

It was previously believed that these mutations happened completely by chance, with no regard for their effects on the organisms. Recently, there have been studies suggesting that these mutations occur in response to environmental challenges. That is to say, they are more likely to occur when they are advantageous to the organism, rather than when they are neutral or disadvantageous.

Categorizing point mutations

- Transition/Transversion categorization
- Transitions: replacement of a purine base with another purine or replacement of a pyrimidine with another pyrimidine
- Transversions: replacement of a purine with a pyrimidine or vice versa.

Nonsense mutation

In genetics, a nonsense mutation is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and in a truncated, incomplete, and usually nonfunctional protein product. It differs from a missense mutation, which is a point mutation where a single nucleotide is changed to cause substitution of a different amino acid. Some genetic disorders, such as thalassemia and DMD, result from nonsense mutations.

Spontaneous mutations

Spontaneous mutations arise from a variety of sources, including errors in DNA replication, spontaneous lesions, and transposable genetic elements. The first two are considered in this section; the third is examined in Chapter 20.

Transitions

All the mispairs described so far lead to **transition mutations,**in which a purine substitutes for a purine or a pyrimidine for a pyrimidine (Figure 16-3). The bacterial DNA polymerase has an editing capacity that recognizes such mismatches and excises them, thus greatly reducing the observed mutations. Another repair system (described later in this chapter) corrects many of the mismatched bases that escapecorrection by the polymerase editing function. Mutation by tautomeric shifts in the bases of DNA. (a) In the example diagrammed, a guanine undergoes a tautomeric shift to its rare enol form (G*) at the time of replication. (b) In its enol form, it pairs with thymine. (c and d) In the next replication.

Transversions

In transversion mutations, a pyrimidine substitutes for a purineor vice versa. Transversions cannot be generated by the mismatches depicted in Figure 16-2. With bases in theDNA in the normal orientation, creation of a transversion by a replicationerror would require, at some point in the course of replication, mispairing of a purine with a purine or a pyrimidine with a pyrimidine. Although the dimensions of the DNA double helix render such mispairs energetically unfavorable, we now know from X-ray diffraction studies that G–A pairs, as well as other purine–purine pairs, can form. In **Frameshift mutations**, Replication errors can also lead to **frameshift mutations.** In the mid-1960s, George Streisinger and his coworkers deduced the nucleotidesequence surrounding different sites of frameshift mutations in the lysozymegene of phage T4. They found that these mutations often occurred at repeated sequences and formulated a model to account for frameshifts in DNA synthesis.

Induced mutation

Induced mutations on the molecular level can be caused by:-

1) Chemicals

- Hydroxylamine NH_2OH
- Base analogs (e.g. BrdU)
- Alkylating agents (e.g. *N*-ethyl-*N*-nitrosourea) These agents can mutate both replicating and non-replicating DNA. In contrast, a base analog can only mutate the DNA when the analog is incorporated in replicating the DNA. Each of these classes of chemical mutagens has certain effects that then lead to transitions, transversions, or deletions. Agents that form DNA adducts (e.g. ochratoxin A metabolites) DNA intercalating agents (e.g. ethidium bromide)
- DNA crosslinkers
- Oxidative damage
- Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns which leads to incorrect base pairing during replication.

2) Radiation

Ultraviolet radiation (nonionizing radiation). Two nucleotide bases in DNA – cytosine and thymine – are most vulnerable to radiation that can change their properties. UV light can induce adjacent pyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer. UV radiation, particularly longer-wave UVA, can also cause oxidative damage to DNA. Mutation rates also vary across species. Evolutionary biologists have theorized that higher mutation rates are beneficial in some situations, because they allow organisms to evolve and therefore adapt more quickly to their environments. For example, repeated exposure of bacteria to antibiotics, and selection of resistant mutants, can result in the selection of bacteria that have a much higher mutation rate than the original population.

Method of Developing Mutant of *Trichoderma* sp. by Using UV Light

- Prepration of conidial suspention (5 day-old *Trichoderma* culture) Spread on Petriplates (contain PDA medium)
- Placed under UV light source for different periods, quartz lamp, & distance (i.e. 10,20,30 etc.)
- Incubated at 25^0C in normal condition
- Comparison of changes with its parent

Impact of Mutation on Morphological Characters

Morphological changes, Biochemical changes, Mycoparasitism are some impacts.

Limitations

1) *Trichoderma* spp. can only be used against specific disease. They are less effective than the fungicides.
2) At present, only few *Trichoderma* spp. are available for use and are available only in few places.
3) This method is only a preventive measure and not a curative measure.
4) *Trichoderma* spp. should be multiplied and supplied without contamination and this requires skilled persons.
5) The shelf life of *Trichoderma* spp. is short. Antagonists, *Trichoderma viride* is viable for four months.
6) The efficiency of *Trichoderma* spp. is mainly decided by environmental conditions.
7) A *Trichoderma* spp. under certain circumstances may become a pathogen.
8) Mutation in *Trichoderma* results in harmful effect on morphological, physiological, biochemical and molecular properties.

CONCLUSION

Induced mutation is one of the most commonly used practices to modify the genetic composition of antagonistic fungi. Mutation on Trichoderma sp. by UV and gamma radiation brought about changes in morphological features like colony diameter, sporulation, dry mycelial weight and enzymes like ß-1,3-glucanase, ß-1,4-glucanase, cellulase and antibiotics like trichodermine, gliotoxin and virindin. Selection of beneficial mutant of biocontrol agent becomes a better option for management of plant pathogens. Adoption of Trichoderma Spp. in biological control in Disease Management is need of time hence extensive studies will be required on interaction of diseases and management practices.

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