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RESEARCH ARTICLE

Isolation of *Agrobacterium tumefaciens* from soil and Optimization of Genomic & Plasmid DNA Extraction

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Abstract

Recent studies of *Agrobacterium tumefacien* species impartants in research the soil born bacterium one of the most impotant species of Agrobacterium genus can cause crown gall in plants. Parasite DNA induced to the host genome resulting in unsightly tumors and changes in plant metabolism. *A. tumefaciens* prompted the first successful development of a biological control agent and is now used as a tool for engineering desired genes into plants. The applied techniques such as isolation, characterization, and molecular aspects of Agrobacterium species, optimization both plasmic and genomicDNA through electrophoresis additionally, we investigated procedure can be completed within few hours accompany with fast DNA extraction materials.

1.1 Introduction

Agrobacterium tumefaciens is a genus of Gram-negative bacteria, gene transfer to cause tumors in plants[1], genetically transforms a vehicle for genetic engineering [2], well known for its ability to transfer DNA [3], between itself and plants; it has an important tool for genetic engineering.

The Agrobacterium species classified into new genera, such as *Ruegeria, Pseudorhodobacter* and *Stappia*, but reclassified as *Rhizobium* species[4]. the soil born bacterium of the most impotant can cause crown gall in both dicotyledonous and monocotyledonous plants[5]. The common use of Agrobacterium as a gene vector for plants has an important plant pathogen[6], Pathogenic strains cause unorganized tissue growth called crown gall[7].

Agrobacterium tumefaciens species to focus on sequence divergence in the vicinity of the genetic boundaries of genomic species[8]. The sequencing of the genomes of several species of Agrobacterium has provided information on the genes and systems involved in pathogenesis, biological control and symbiosis[9] this group knows as of plant-associated microbes.

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1.2 Uses in biotechnology

Ever since, geneticists have found various genetic events at which some genetic elements are capable of moving around the genome actively or passively. These genetic elements now referred to as mobile genetic elements (MGEs)[10]. The ability of Agrobacterium to transfer genes to plants and fungi is used in biotechnology, A modified Ti or Ri plasmid can be used[11]. One distinct feature of the infection relies on the presence of the Ti plasmid[12], which induces formation of tumors on plant stems, and the Ri plasmid in A. rhizogenes, which is responsible for hairy root formation[13]. Agrobacterium does not infect all plant species, but there are several other effective techniques for plant transformation including the gene gun.

Agrobacterium tumefaciens, the cause of the economically important disease, crown gall, has also been studied for years because of its remarkable biology. The mechanism this bacterium uses to parasitize plant tissue involves the integration of some of its own DNA into the host genome resulting in unsightly tumors and changes in plant metabolism. *A. tumefaciens* prompted the first successful

development of a biological control agent and is now used as a tool for engineering desired genes into plants.

1.3 Identification of Agrobacterium

Agrobacterium tumefaciens is a member of the family *Rhizobiaceae*. grow aerobically, without forming endospores. The cells are rod-shaped and motile, having one to six peritrichous flagella. Cells are 0.6-1.0µ diameters 1.5- 3.0µ Larges and may exist singly or in pairs[14]. In culture on carbohydrate-containing media, cells produce large amounts of extracellular polysaccharides, giving colonies a voluminous, slimy appearance. species, *A. tumefacians*, *A. radiobacter*, and *A. rhizogenes*, within the new taxon: *Agrobacterium tumefaciens.*

1.4 Isolation of agrobacterium's

*A. tumefaciens*can be isolated from gall tissue, soil or water[21]. Optimal gall tissue for isolation is white or cream-colored from a young, actively growing gall. The gall should be washed or surface sterilized using 20% household bleach, and rinsed several times in sterile water. Cut a few samples from different parts of the white tissue of the gall, and further dividesamples into small pieces. Place these pieces into a culture tube containing sterile distilled water or buffer, vortex and allow standing for at least 30 minutes. Using an inoculating loop, streak this suspension on Medium and incubate at 25-27 °C. Different strains will grow at different rates[15].

2. Materials and Methods

2.1 Isolation of Agrobacterium from soil

The soil materials collected from field of Athner village of Betul District M.P., India. The Agrobacterium, Firstly we isolated from soil, prepared dilution series and cultured of the sample on YEMA media[16] cultivated in modified medium containing 3.0 g/L of $(NH_4)_2SO_4$, 0.1 g/L of KCl, 0.5 g/L of K_2HPO_4 and $MgSO_4$-$7H_2O$,[17]. After 5-7 days we got some colonies of pink color, Colonies of pink color shows in figur1, confirmation of Agrobacterium. After that we prepared pure culture of Agrobacterium then prepared broth.

2.2. Identification & characterization of Agrobacterium by staining process

Aseptically obtain the loopful of broth and agar cultured, mix the amount of culture into a drop of water on each separate glass slide prepare a mixture that is only slightly milky Pinkcolour smear in form of round spread on center zone of slides . Allow the slide to air dry before heat fixation. After heat fixation, the slide is ready to stain. Apply gram staining dyes, cover the entire smear with stained, examine by microscope using the oil immersion objective lance show in figure 2.

2.3 Extraction of Genomic and Plasmid DNA

Plasmid are extra chromosomal DNA molecules that exist inside bacterial cells they replicate independently of the bacterial genome and segregate to the progeny when a bacterial cell divides so they can be maintained indefinitely in a bacterial lineage and are also called as replicons many plasmids are circular DNA molecules but others are linear The numbers of copies of a plasmid in a cell varies from one plasmid to next depending on the mechanism by which replication is regulated. Genomic DNA is the total DNA Extracted From the cells of organisms[18].

The DNA will be isolated by the alkali lysis method[19]. In this method bacterial suspension is the first incubated with the GTE mix. It is containing glucose, Tris-HCl and EDTA. The EDTA chelates divalent metals primarily magnesium causing removal of Mg^{++}ion which destabilize the cell membrane and inhibit DNAse. glucose is mainly added to maintain and prevent the tris buffer from bursting the cells after this the cells are exposed to the strongly anionic detergent of a high pH causing the opening of the cell wall, denaturing the chromosomal DNA[20]. Through plasmids DNA is also denaturation but because the strands are intertwined these do not separate. The plasmids DNA thus renatures rapidly when pH is returned to neutral. SDS is detergent which is use to lysis the bacterial cell wall denatured protein and chromosomal DNA.These are precipitating by using potassium acetate. The isolated DNA is subjected to electrophoresis. Electrophoresis is a technique for separation or resolving molecules in a mixture under the influence of electric field where the migration rate is determined by charge: mass ratio. Electrophoresis through agarose gel is the standard methods used to separate, identify and purity DNA fragments. Agarose gel is usually run in a horizontal configuration in an electric field of constant straight and direction. Visualize the gel in an UV illuminator show in figure 3.

Fig.1:- Isolation of Agrobacterium colonies from soil on YEMA Media.

Fig.2:- Microscope view Agrobacterium

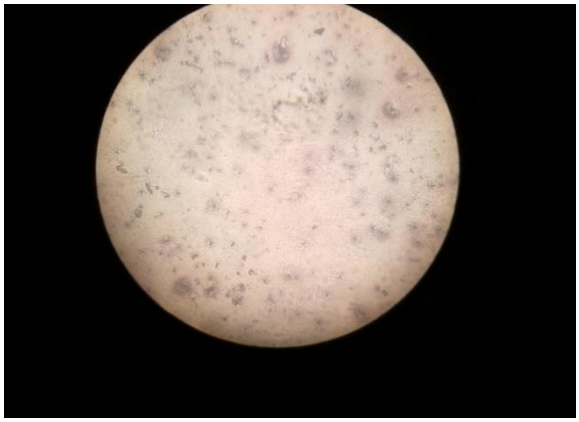
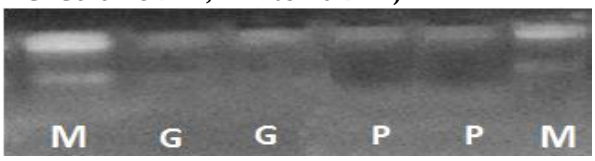


Fig.3:-Agarose gel estimation of DNA (M= Marker, G=Genomic DNA, P= Plasmid DNA)



3. Results and Conclusion

In the presence research investigation the crown gall caused by *A. tumefaciens*is becoming a big threaten to nursery and fruit production. It's may be a sensitive and specific detection method is needed in early pathogen diagnosis for the symptomless host plant due to the specific infection mechanism. As expected, none of them could produce the positive band compared to the *A.tumefaciens*, which is regarded as the most dominant species in soil environment. Even the *Agrobacterium rhizogenes*and *Agrobacterium vitis* species also could not amplify the positive band but both species are closely relative to *A. tumefaciens* according to their phytopathogenic characteristics and could be caused crown gall on other plants[5]. Latent diagnosis is more important since the symptomless seeding and soil are the main sources of this disease, and according to the specific invasion mechanism it is hard to control in case the symptom emerged. This result gives us important information that control measures should be carried out immediately in order to prevent uncertain loss. We are want to introduce advance Methods of DNA Extraction from Bacterial cells, additionally our procedure can be completed within 6 hours accompany with fast DNA extraction materials[22]. It is very convenient and easy to be developed in actual diagnosis.

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