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ORIGINAL RESEARCH ARTICLE

Analysis of Induced Mutation in Rhizobium Using Different Azo Dyes by RAPD-PCR

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ABSTRACT

Dyes make the world more beautiful through coloured substances, but on the other hand they represent a serious pollution problem for the environment. The ecotoxicological and toxicological problems due to discharge of textile waste water in water bodies have been one of the most important water pollution. This textile water are rich in dyes such as acidic dyes, azoic dyes, sulfur dyes, etc. These dyes are carcinogenic, mutagenic, Teratogeneic toxic to microorganisms and plants. This high concentration of dyes reduces the soil fertility by affecting the beneficial microbes in soil. Among these beneficial microbes *Rhizobium japonicum* is one of the most important nitrogen fixers. The dye components which are getting sediment in the surface of the soil can induce mutation in Rhizobium. The objective of this study is to analyze mutation which was induced by methyl orange, an azo dye. The *Rhizobium* was isolated from the root nodules of ground nut plant and it was identified by morphological analysis. The *Rhizobium* culture was mass cultured in Yeast Extract Medium (YEM) broth and it was exposed to different concentration of azo dyes. After the proper growth, the DNA was isolated from the strains and it was conformed and amplified by PCR for the analysis of induced mutation. The amplification was done by Agarose Gel Electrophoresis and the percentage of mutation was analyzed by Dendogram analysis.

Key words: *Rhizobium*, Azo dyes, Mutation and RAPD-PCR.

1. INTRODUCTION

The textile industries are to satisfy the ever – growing demands in terms of quality, variety, fastness and other technical requirements. However, a recent study was conducted under the National Biodiversity Strategy and Action Plan (BSAP) has revealed that chemical colors have all but wiped out India's wonderful vegetable dyes. Indian industry produces every type of dyes and pigments. The production of dye stuff and pigments in India is close to 80,000 tonnes. India is the second largest exporter of dye stuff and intermediates developing countries after china. The textile industry accounts for the largest consumption of dye stuff at nearly 80%. During the last decade, environmental issues associated with dye stuff production and application has grown significantly and indisputably among the major driving force affecting the textile dye industry today. Textile waste contains high levels of dyes, their intermediates and impurities. Most of the dyes used in textile industries suspected that, they have toxicity. The removal of dye from textile effluents is one of the most significant environmental problems. Dyes are used in large quantities in many industries including textile, leather, cosmetics, paper, plastics, pharmaceuticals, food, etc. New dyes are continuously being developed to meet the demands of new technology, new fabrics, and advances in dyeing equipment and to overcome the serious environmental concerns associated with some exiting dyes.

Two major source of dyes release into the environment are the textile and dye stuff manufacturing industries [1]. Synthetic dyes are not uniformly susceptible to biodegradation in conventional biological waste water treatment process because of their resistance to microbial azo dyes, which are used extensively in many industries are the largest class with a wide verity of colours and structure. Synthetic dyes are used extensively in textile and leather dyeing, paper

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printing, colour photography and as additives in petroleum product with the growing use of a variety of dyes [2]. Chemical, biological and physicochemical methods including reverse osmosis have been used for colour removable all of which however are relatively expensive [3]. Azo dyes are one of the oldest industrially synthesized organic compounds and under anaerobic condition can be converted to aryl amines which are potentially more toxic than the parent compound [4, 5]. Azo dyes are designed to be recalcitrant under typical product service conditions. Their toxicity to microorganisms that make biological treatment difficult [6].

The presence of an azo linkage in aromatic compounds makes them highly important in dye stuff industry, pharmacy and dosimetry [7]. Electrochemical reactions of azo components usually occur in 2 $^{e-2+}$ process to give hydrazo products. Azo dyes are the largest class of dyes commercially used in the textile industries [8]. Most of these components are highly resistance to microbial attack and are therefore hardly removed from effluent by conventional biological waste water treatments, such as activated sludge [9].This acid dye is used for wool and silk. It imparts an orange colour as the name suggests though the colour is not fast to light. It is used acid alkali titration as an indicator and gives a yellow colour with alkali and pink with acid.

There is considerable evidence that mutations induced in polygenic traits and that there is a genetic gain under selection. Irrespective of mutagens used seed treated plants usually come out the ressessive mutans in the second (M2) or third (M3) generation after the treatment [10] and [11] M2 generation, macromutations may be observed particularly following radiation treatment. The macromutations are usually undesirable due to accompanying genetic instability. Micromutations that alter quantitatively inherited characters are more useful to the breeders .Since they are least deleterious although they are more difficult to detect. RAPD (Random Amplified Polymorphic DNA) technique is a powerful tool for genetic studies. However, its use for genetic population analysis may be limited by the laborious procedure involved in the extraction of genomic DNA from large sets of samples. Recently, procedures such as boiling have been used to promote cell lysis and detect pathogens in the plants tissues [12] and [13].The molecular markers are extensively used in germplasm characterization, finger printing, genetic analysis, linkage mapping and molecular breeding. These markers are also used in identification of possible so monoclonal variants at an early stage of development which is considered very useful for quality control in plant tissue culture, transgenic plant production and in the introduction of variants [14].

Random Amplified Polymorphic DNA analysis using PCR in association with short primers of arbitrary variation among individuals. The advantages of this technique are (1) A large number of samples can be quickly and economically analyzed using only micro qualities of materials. (2) The DNA amplicons are independent from the ontogenetic expression many genomic regions can be sampled with a potentially unlimited number of markers [15]. The present study is thus aimed at studying the mutagenic potential of the locally available and used textile dyes. Most of these dyes have not been characterized regarding their chemical nature, purity, possible toxicity or their impact on health and the environment [16,17]. Assessment of genotoxicity of is therefore of almost importance [18,19,20].

2. MATERIALS AND METHODS

2.1. Isolation and identification of *Rhizobium japonicum*

The root nodules were surface sterilized by mercuric chloride and crushed nodules were mixed with sterile distilled water. It was serially diluted and spread over the Yeast Extract Mannitol (YEM) agar plates after incubation time mucoid colony growth was observed on the agar plates, the grown colonies were identified as *Rhizobium japonicum* by Staining techniques, motility test, plating on selective medium and biochemical tests.

2.2. Isolation and estimation of DNA from azo dye induced mutants

The isolated *Rhizobium japonicum* culture was grown in YEM broth and different concentration (1ppm, 2ppm, 3ppm, 4ppm) of Methyl orange and control was maintained with adding dye. Fine growth was observed in control and the growth of *Rhizobium japonicum* decreased when the concentration of dye was increased. The DNA was isolated by phenol chloroform extraction method and it was confirmed by agarose gel electrophoresis method.

2.3. Amplification of DNA by PCR for induced genetic variation analysis

The DNA samples were amplified using decamer primers which were complementary to short tandom repeat present DNA after 40 cycles of polymerase chain reaction the samples were

loaded in 1.5% agarose gel. Discreate amplified bands were observed under UV-transilluminator and the gel was photographed and the image was applied in non-linear dynamic software and the variation between amplified bands was observed by presence and absence of DNA fragments. The dendogram was constructed based on UPGMA cluster analysis according to dice square co-efficiant method. The dendogram results was observed that from the control strain 1ppm dye have altered the DNA at an average of 0.53 nucleotides, similarly from the 2ppm dye the culture exposed to 3ppm dye have got altered 0.36 nucleotides from the control *Rhizobium japonicum* and at the highest 4ppm dye have altered 0.91 nucleotides from the control. From the results, it was clearly observed that the increasing in concentration of dye will leads to high rate of mutation in bacteria.

Table 1: Visualization of DNA in agarose gel

S. No	Concentration of DNA	Amount of concentrations of DNA
1	Control	82µg/ml
2	1ppm	73µg/ml
3	2ppm	68µg/ml
4	3ppm	59µg/ml
5	4ppm	45µg/ml

Figure 1: Confirmation of DNA on Agarose gel electrophoresis

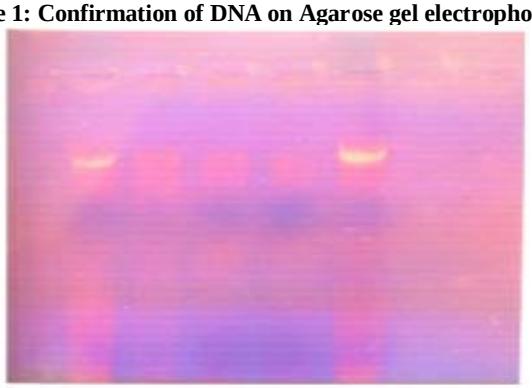
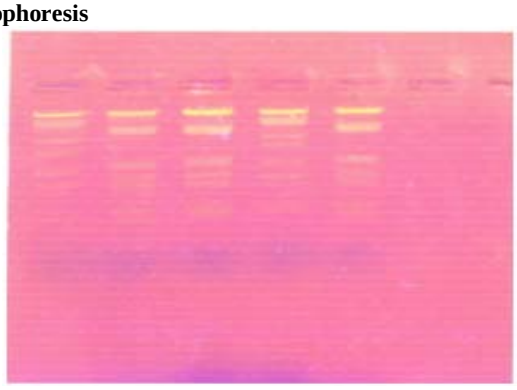


Figure 2: Confirmation of PCR amplified DNA on Agarose gel electrophoresis

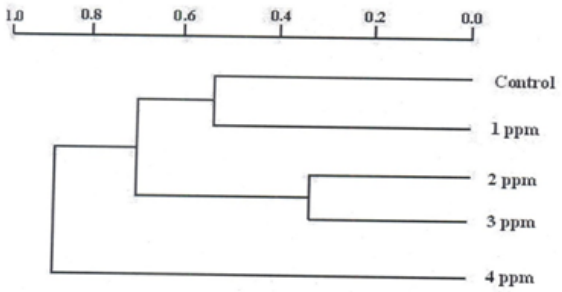


2.4. Visualization of DNA in agarose gel

Agarose is a linear polysaccharide made up of a basic repeat unit of agaobiose, which comprises alternative units of D-Galactose and 3,6 anhydro galactose. Agarose gel electrophoresis is a technique used to resolve DNA fragment on the basis of their molecular weight smaller fragments migrate faster then larger ones. The distance migrated on the gel varies inversely with the logarithm of the molecular weight. Calibrating the gel using known size standards and comparing the migration distance of the unknown fragment.

The DNA samples were amplified using decamer primers which were complementary to short tandom repeat present DNA after 40 cycles of Polymerase chain reaction the samples were loaded in 1.5% agarose gel. Discreate amplified bands were observed under UV-transilluminator and the gel was photographed and the image was applied in non-linear dynamic software and the variation between amplified bands was observed by presence and absence of DNA fragments. The dendogram was constructed based on UPGMA cluster analysis according to dice square co-efficient method. The dendogram results was observed that from the control strain 1ppm dye have altered the DNA at an average of 0.53 nucliotides, similarly from the 2ppm dye the culture exposed to 3ppm dye have got altered 0.36 nucliotides from the control *Rhizobium japonicum* and at the highest 4ppm dye have altered 0.91 nucliotides from the control. From the results, it was clearly observed that the increasing in concentration of dye will leads to high rate of mutation in bacteria.

Figure 3: Analysis of genetic variation by Dentogram



3. RESULTS AND DISCUSSION

The ecological and toxicological problems due to discharge of textile waste water in water bodies have been one of the most important water pollution. This textile water are rich in dyes such as acidic dyes, azoic dyes, sulfur dyes, etc. This dyes are carcinogenic, teratogenic toxic to microorganisms and plants. This high concentration of dyes reduces the soil fertility by affecting the beneficial microbes in soil. Among these beneficial microbes, *Rhizobium japonicum* is one of the most important nitrogen fixers. The dye components which are getting sediments in the surface of the soil can induce mutation in *Rhizobium japonicum*. The objective study is to analyze mutation which was induced by methyl

orange an azo dyes. The *Rhizobium japonicum* was isolated from the root nodules of ground nut plant and it was identified by morphological and biochemical analysis. The *Rhizobium japonicum* cultures were mass cultured in YEM broth and it was exposed to different concentration of azo dyes. After the proper growth the DNA was isolated from the strains and it was confirmed and confirmed and it was amplified by PCR for the analysis of induced mutation. The amplification was confirmed by AGE and the percentage of mutation was analyzed by dendogram analysis. The dendogram results, it was observed that the control strain 1ppm dye have altered the DNA at an average of 0.53 nucliotides, similarly from the 2ppm dye the culture exposed to 3ppm dye have got altered 0.36 nucliotides from the control. The *Rhizobium* and at the highest 4ppm dye have altered 0.91 nucliotides from the control. The results it was clearly observed that the increasing in concentration of dye will leads to high rate of mutation in bacteria [21, 22].

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