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Aerobic biodegradation of Acid Blue-9 dye by *Bacillus fermus* Isolated from *Annona reticulata*

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HIGHLIGHTS

- Isolation of an endophytic bacteria from *Annona reticulata*.
- Identification of endophytic bacteria as *Bacillus fermus*, a potential aerobic degrader of Acid Blue-9.
- Standardization of conditions for optimal degradation of Acid Blue-9 by *Bacillus fermus*.
- Deliberation of possible biodegradation pathway for Acid Blue-9 by *Bacillus fermus*.
- Cytogenotoxicity test indicating reduced chromosomal damage in degraded samples in comparison to Acid Blue-9 dye.

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ABSTRACT

A dye being reluctant to degradation due to their structural complexity requires a cost-effective and an eco-friendly technique of degradation. Present study, *Bacillus fermus* isolated from *Annona reticulata*, gram positive bacteria was employed for the degradation of Acid Blue-9 dye. Minimal salt media consisting of Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl, $MgSO_4$ with varying concentration of dye and carbon source was used for the study. Among various carbon sources used, sucrose proved to be an efficient carbon source yielding 97% decolorization. Sucrose concentration of 3g/L showed maximum degradation along with 3% v/v of inoculum concentration. The decolorization was confirmed from the results obtained by UV–visible spectrophotometer at 579 nm. The possible biodegradation pathway was obtained from the analysis of Liquid Chromatography–Mass Spectrometry techniques. It was also observed that degraded product was known to induce lesser chromosomal aberrations in comparison to untreated Acid Blue-9.

1. Introduction

Colorant occupies a central role in human lifestyle. Amplification in dye consumption is seen as a result of rapid industrialization for the fulfillment of ever-increasing need. Dyes are chemical compounds that are routinely used in cosmetics, textile, leather, printing, and food. They are released in higher quantity in industrial effluents (Alhassani et al., 2007). Inefficacious release of spent effluent has led to contamination of the ecosystem (Nigam et al., 1996). Bioaccumulation of these dyes due to their obduracy in the environment introduces the eco-toxic hazards which eventually enter food chain and negatively influence human beings. Synthetic dyes are known to be toxic, carcinogenic and mutagenic (Clemmensen et

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al., 1984; Sanmuga et al., 2016) in nature. Eco toxicity of these dyes and their impact on the environment is studied in detail with reference to neutral red dye (Farzana et al., 2015) which suggest a significant increase seen in mutagenesity. Aquatic as well as terrestrial components of eco-system are badly influenced by effluents. The intense color developed by these dye effluents impede the aquatic environment by reducing penetration of light, gas solubility and interfere in photosynthesis (Saranaik and Kanekar, 1995).

Conventional physico-chemical methods partially leads to degradation and are incompetent in conversion to CO_2 (Lin et al., 2011; Lin and Peng, 1994). Various methods like absorption, flocculation, and chemical precipitation have certain disadvantages like complex structural set-up, investment on chemicals, huge power consumptions and release of huge quantity of unpleasant toxic sludge (Mcmullan et al., 2001). In contrast, microbial remediation is proved to be more lenient by releasing environmentally benign components, less sludge and being cost effective (Walker, 1970). Microbial degradation is highly effected by the change in pH and thus highly alkaline pH of sludge reduces the microbial activity. Those microorganisms with alkalophilic nature can sustain such condition without any damage. Substantial presence of microorganism and reports based on their ability to degrade dyes makes them a promising tool for remediation (Farzana et al., 2015). Apparently, the development of novel approach for degradation of these toxic dyes using non-pathogenic strains of bacteria with concern to human beings is of utmost importance. Bacterial endophytes being a part of our environment have proved to be potentially beneficial in restoring balance in many ways. They act as bio control agents against various diseases. Bacterial endophyte from *Theobroma cacao* has been reported to control cacao disease (Rachel et al., 2011). Studies also suggest bacterial degradation, decolorization and detoxification of effluents from textile industries (Swapnil et al., 2011). Scientific reports also suggest the comparison studies between the free cell and immobilized cells to study the efficiency of bacterial degradation using *Burkholderia vietnamiensis* (Ying et al., 2012).

According to our knowledge, there exists no published work on the biodegradation of Acid Blue-9 dye using an endophytic bacterium isolated from a plant source. Thus the present study makes an attempt to understand the probable pathway of the degraded product and to stabilize various parameters for the optimal degradation by the bacteria. Also this study reports an isolation of the bacterial endophyte, the required conditions and parameters for the optimal degradation and the probable pathway in the degradation of Acid Blue-9. Further it includes the following objectives: (1) bacterial endophytic isolation and identification (2) variation in the parameters to understand the optimal levels of degradation (3) confirmation of the degradation and to understand the probable pathway involved in the same.

2. Materials and methods

2.1. Chemicals and culture media

Minimal salt basal medium was employed in assessment of degradation studies. Media composed of 6 g/L, Na_2HPO_4: 3 g/L, KH_2PO_4: 5 g/L, NaCl: 2 g/L, NH_4Cl: 0.1 g/L, $MgSO_4$ salts were purchased from Merck Pvt. Ltd., India. All salts were dissolved in double distilled water in preparation of minimal media. Luria–Bertani medium (LB medium) used for isolation of bacterial endophytes was purchased from HiMedia Laboratory, India. Acid Blue-9 dye was purchased from LobaChemie. Pvt. Ltd., India.

2.2. Explant collection and sterilization

Annona reticulata, bark Sample was collected in the month of January 2016 (winter season). This host plant is located in Udupi town (altitude: 25 m; latitude: 130 31′N; longitude: 740 74′E) India. Average annual temperature is 27 °C and precipitation averages 4465 mm. The explant were collected in clean polythene bags, cleaned in tap water and blot dried and immediately, Prior to sterilization the explant was held under running tap water for about a minute to remove any external deposited surface contaminants. The explant weighing 2 g was initially treated with 0.1%v/v Tween 20 for 2 min. It was further treated with 1%v/v sodium hypochlorite for 3 min and later rinsed in distilled water for 5 min. This was followed with 70%v/v ethanol wash for 3 min and finally washed with sterile distilled water.

2.3. Isolation and identification of endophytic bacteria from explant

Luria–Bertani plates were used for isolation of endophytic bacteria (Bhore et al., 2010: Chen et al., 2012). Sterilized bark tissue of *Annona reticulata* was ground to slurry using 10 ml of sterile distilled water. Sterile mortar and pestle was used for grinding. Spread plate technique on Luria–Bertani (LB) agar plates were carried out and these plates were incubated at 37 °C for 24 h. Colonies were screened for the ability to degrade dyes on Luria–Bertani plates. Based on the zone of clearance the bacterial isolates are selected for the degradation studies. Molecular identification was performed using isolation of genomic DNA by amplifying 16S rRNA region. Primers for 16S rRNA gene 8F 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 1490-5′-GAC TTA CCAGGG TAT CTA ATC C-3′ (Sigma) was used. Nucleotide database was searched with the sequences obtained with NCBI (National Centre for Biotechnology Information) BLAST (Blastn) tool: http://www.ncbi.nlm.nih.gov/BLAST. After performing multiple alignment of the sequence, a Neighbor joining Phylogenetic tree was constructed.