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Environmental Bioremediation of Dyes by *Pseudomonas aeruginosa* ETL-1 Isolated from Final Effluent Treatment Plant of Ankleshwar

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Abstract Economical and bio-friendly approaches are needed to remediate dye-contaminated wastewater from various industries. In this study, a novel bacterial strain capable of decolorizing triarylmethane dyes was isolated from a textile wastewater treatment plant in Ankleshwar, Gujarat, India. The bacterial isolate was identified as *Pseudomonas aeruginosa* ETL-1 and was shown to decolorize three triarylmethane dyes tested within 24 h with color removal in the range of 72% to 96%. Decolorization efficiency of the bacterium was a function of operational parameters (aeration, dye concentration, temperature, and pH) and the optimal operational conditions obtained for decolorization of the dyes were: pH 7-8, 35°C and culture agitation. Effective color removal within 24 h was obtained at a maximum dye concentration of 50 mg/l. Dye decolorization was monitored using a scanning uv/visible spectrophotometer which indicated that decolorization was due to the degradation of dyes into non-colored intermediates. Phytotoxicity studies carried out using *Triticum aestivum*, and *Lens esculenta* revealed the triarylmethane dyes exerted toxic effects on plant growth parameters monitored. However, significant reduction in toxicity was obtained with the decolorized dye metabolites thus, indicating the detoxification of the dyes following degradation by *Pseudomonas aeruginosa* ETL-1.

Keywords: *Pseudomonas, triarylmethane dyes, Ankleshwar, bioremediation*

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1. Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life [30]. Reactive dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. The three most common groups are azo, anthroquinone and phthalocyanine [31]. Worldwide, dye wastewater has become one of the main sources of severe pollution problems due to the greater demand for textile products and the proportional increase in production and applications of synthetic dyes [1]. It is estimated that over 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually worldwide [47]. In the textile industry, up to 200,000 tons of these dyes are lost to effluents every year during dyeing and finishing operations as a result of inefficiency in the dyeing process [17,53]. Unfortunately, most of these dyes escape conventional wastewater treatment processes and persist in the environment as a result of their high stability against light, temperature, water, detergents, chemicals, and microbial attack [42]. Notwithstanding, industries are required to eliminate color from their effluents containing dyes, before disposal into water bodies, due to environmental legislation [7]. Among the many different groups of synthetic dyes, triarylmethane (also called triphenylmethane) dyes are one of the most commonly used in the textile industries. Their usage constitutes about 30%-40% of the total consumption of dyes [9], and they are applied extensively on nylon, cotton, wool, and silk. They are also used for coloring food, oils, fats, waxes, varnishes, cosmetics, paper, leather, and plastics [34] as well as for staining specimens in bacteriological and histo pathological processes. With dye tinctorial value usually high, less than 1 ppm of dye in water produces obvious coloration [48], and the extensive use of these dyes have resulted in highly colored effluents that may affect gas solubility in water bodies [18] and significantly decrease photosynthetic activity in aquatic life because of reduced light penetration. In addition to their visual effect, triarylmethane dyes are generally believed to be toxic and carcinogenic or prepared from other known carcinogens [12,39]. Several reports have also shown that textile dyes and effluents have toxic effects on plants which perform important ecological functions such as providing a habitat

for wildlife, protecting soil from erosion, and providing the organic matter that is so significant to soil fertility [14]. Consequently, it is pertinent to develop efficient treatment strategies for removal of color from dye wastewater. Various physicochemical methods, such as adsorption on activated carbon, electro coagulation, flocculation, froth flotation, ion exchange, membrane filtration, ozonation, and reverse osmosis have been used for decolorization of dyes in wastewater [47,49]. However, these methods are less efficient, costly, of limited applicability, and produce wastes, which are difficult to dispose of [32]. On the contrary, biological processes provide a low-cost, environmentally benign, and efficient alternative for the treatment of dye wastewater [15]. Decolorization by biological means may take place in two ways: either by adsorption (or biosorption) on the microbial biomass or biodegradation by the cells [52]. Biosorption involves the entrapment of dyes in the matrix of the adsorbent (microbial biomass) without destruction of the pollutant, whereas in biodegradation, the original dye structure is fragmented into smaller compounds resulting in the decolorization of synthetic dyes. Several researchers have described the use of microorganisms as biosorption agents in the removal of pollutants from wastewater [44,48,49]. However, due to operational ease and facile adaptability of microorganisms to a given set of conditions, the biodegradation mechanism is considered efficacious in comparison to biosorption for treatment of dye wastewater [23]. Over the past few decades, numerous microorganisms have been isolated and characterized for degradation of various synthetic dyes, but most of the reports have dealt mainly with decolorization of azo dyes [4,5,27]. There is a dearth of information regarding the degradation and detoxification of triarylmethane dyes by microbial systems despite their increased use by the textile industry. Hence, the isolation of potent species that have the capability for degradation and detoxification of triarylmethane dyes is of interest in the biotechnological aspect of dye effluent treatment. In this study, a bacterial strain, *Pseudomonas aeruginosa* ETL-1, capable of decolorizing triarylmethane dyes was isolated from textile industrial wastewater using the selective enrichment method. The effects of various parameters (such as culture agitation, initial dye concentration, pH, and temperature) on dye decolorization by the bacterial strain were investigated and the toxicity of the products formed after decolorization was determined using plant assay.

2. Materials and Methods

2.1. Chemicals

The triarylmethane dyes (basic violet 14, basic violet 3, and acid blue 90) used in this study were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). The stock solutions of each dye were prepared by membrane filtration. All other chemicals used were of analytical grade.

2.2. Bacteria and Growth Medium

The bacterial strain used was isolated from dye wastewater obtained from final effluent treatment plant (FETP) of Ankleshwar, Gujarat, India. The principle of sequential selective enrichment batch culture for selection of dye decolorizing bacteria was employed in synthetic wastewater medium (SWM) with Basic Violet 3 as the carbon source. The basic composition of the synthetic wastewater medium was (g/L); $(NH_4)_2SO_4$ 0.28, NH_4Cl 0.23, KH_2PO_4 0.067, $MgSO_4 \cdot 7H_2O$ 0.04, $CaCl_2 \cdot 2H_2O$ 0.022, $FeCl_3 \cdot 6H_2O$ 0.005, NaCl 0.15, $NaHCO_3$ 1.0, and 1 mL/L of a trace element solution containing (g/l); $ZnSO_4 \cdot 7H_2O$ 0.01, $MnCl_2 \cdot 4H_2O$ 0.1, $CuSO_4 \cdot 5H_2O$ 0.392, $CoCl_2 \cdot 6H_2O$ 0.248, $NaB_4O_7 \cdot 10H_2O$ 0.177, and $NiCl_2 \cdot 6H_2O$ 0.02. The textile wastewater used for isolation of dye-decolorizing bacteria was acclimatized for 8 weeks prior to transfer into 250mL Erlenmeyer flasks containing 100mL SWM. After incubation of the flasks, a mixed culture that showed quick and stable decolorization activity was transferred to newly prepared SWM. After five successive transfers, it was plated on SWM agar containing 20 mg/l of each dye and incubated at 30°C for 5 days. Bacterial colonies around which clear zones expanded quickly were picked for further studies and designated as ETL 1-5. To check for the dye-degrading potential of each bacterial isolate, preliminary batch experiments were carried out using sterile 250mL Erlenmeyer flasks containing 100 ml of SWM spiked with dye, after which the solution was inoculated with freshly grown bacterial cells. The final pH was 7.2. The bacterial isolate that showed the highest ability to degrade the triarylmethane dyes was selected and used for subsequent investigations. The selected bacterium (ETL-1) was characterized and identified as *Pseudomonas aeruginosa* using Gram stain, spore test, motility test, and a battery of biochemical and physiological tests as described by Vanderzannt and Splittstoesser and Cheesbrough and with reference to the Bergey's Manual of Determinative Bacteriology [19].

2.3. Identification of Bacteria Using 16S rRNA

The total genomic DNA of ETL-1 was isolated using Promega WIZARD® Genomic DNA Purification kit. The forward primers, FD1 (5'- AGAGTT TGATCC TGGCTCAG -3') and the reverse primer, RD1 (5'- AAGGAGGTGATCCAGCC -3') were used to amplify the 16S rRNA sequence of ETL-1. PCR conditions included an initial denaturation for 5 min at 95°C followed by 30 cycles of 1 min at 95°C, 1min at 50°C and 2 min at 72°C. The amplicons were purified and sequenced. The 16S rRNA sequence of MPS-2 was analyzed using basic local alignment search tool.

2.4. Phylogenetic Analyses of ETL-1 16S rRNA Gene Sequences

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 [31]. The evolutionary history was inferred using the neighbor-joining method [33]. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxonomy analyzed [35]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxonomy clustered together in the bootstrap test (500 replicates) is shown next to the

branches [35]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [36] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1420 positions in the final dataset. This tree was rooted with gram negative bacteria *Eschericia coli* strain K12 MG1655.

2.5. Batch Decolorization Operation

The decolorization of the triarylmethane dyes was studied in 250 ml erlenmeyer flasks containing 100 ml of SWM and bacterial biomass in a batch mode. Each flask was inoculated with 2 ml of freshly grown *Pseudomonas aeruginosa*. The inoculum size was adjusted at optical density 1.0 at λ = 620nm (1.50 × 107 cells/mL) and incubated under shaking (150 rpm) and static conditions at 30°C. To evaluate the effects of other operational factors on the efficiency of color removal, the batch decolorization experiments were carried out at different initial dye concentrations (1-100 mg/l), temperatures (15°C-45°C) and initial pH (4-10) under shaking incubation condition. Thereafter, optimal conditions of 35°C and pH 7 and initial dye concentration of 50mg/l were used in subsequent experiments under shaking condition. Three types of control were used: uninoculated sterile control, heat-killed control, and sodium azide (0.1% w/v) amended control. The first indicated the effect of medium components on decolorization, and the latter two showed adsorption of dyes on cells. After incubation, aliquots (5 ml) were taken and centrifuged at 8,000 rpm for 15 min to separate the bacterial cell mass and obtain a clear supernatant, which was used to measure the absorbance of culture samples at the maximum absorption wavelength (λmax) of the respective dyes using a scanning spectrophotometer (Shimadzu UV-1800, Japan). Residual dye concentration of samples was then obtained from a calibration curve of dye concentration versus absorbance prepared for each dye. Decolorization activity was determined by using adsorption (A) and dye removal by living biomass (R) which were calculated according to the following formulae (11):

$$A(\%) = \left[\frac{C_0 - C_1}{C_0}\right] \times 100\%$$

$$R(\%) = \left[\frac{C_0 - C_{1L}}{C_0}\right] \times 100\%$$

Where C_0--the concentration (mg/L) of dye in control sample, C_1--the residual concentration (mg/l) of dye in culture samples with killed or sodium azide treated cells, C_{1L}--the residual concentration (mg/l) of dye in samples with living biomass. Enumeration of bacterial counts in culture flasks was carried out on plate count agar (Hi-Media, India) after 10-fold serial dilution of culture samples using the spread plate method. Viable cell counts obtained after incubation for 24 h at 35°C were expressed as colony-forming units per mL (CFU/ml).

2.6. Toxicity Study

Phytotoxicity studies were carried out with 50 mg/l of each dye and its extracted metabolites using seeds of *Triticum aestivum*, and *Lens esculenta* with SWM as control. The degradation metabolites of each dye extracted in ethyl acetate were dried and dissolved in water to form the final concentration of 50mg/L for phytotoxicity studies [12]. The seeds were surface sterilized with 1.2% sodium hypochloride solution to discourage fungal growth. Fifteen seeds of each plant species were placed in each petri dish in sets and watered separately with 5 ml samples of each dye and its degradation product per day. The Petri dishes were kept in the dark and observed for germination. Seeds with radicle (> 1mm) were considered germinated [16]. The germinated seeds were then exposed to day and night cycle length of 10/14 h, respectively, with a temperature regime of about 28 ± 2°C. The length of plumule (shoot) and radicle (root), and the germination rate (%) were recorded after 7 days.

2.7. Statistical Analysis

Data presented are means of three replicates (±SE) obtained from three independent experiments. Data were analyzed using analysis of variance (ANOVA), with the Dunett post hoc test to check for interactive effects between factors [2]. The significance level was set at 5%.

Table 1. Color removal efficiency of dye-degrading bacteria isolated from industrial wastewater

Isolate	Basic violet 14		Basic violet 3		Acid blue 90	
	Decolorization due to reduction (%)	Decolorization due to adsorption (%)	Decolorization due to reduction (%)	Decolorization due to adsorption (%)	Decolorization due to reduction (%)	Decolorization due to adsorption (%)
ETL-1	74.0 ± 0.5	2.5 ± 0.8	45.0 ± 0.8	6.8 ± 0.8	30.2 ± 0.5	6.2 ± 0.2
ETL-2	52.2 ± 0.8	6.0 ± 1.2	40.5 ± 0.2	6.4 ± 1.4	35.0 ± 0.9	5.8 ± 0.8
ETL-3	65.5 ± 0.3	3.5 ± 0.6	52.0 ± 1.4	7.6 ± 0.8	33.8 ± 0.7	3.5 ± 0.7
ETL-4	90.4 ± 0.5	ND	78.6 ± 0.5	0.8 ± 0.2	82.6 ± 0.5	ND
ETL-5	48.8 ± 0.7	5.8 ± 0.7	34.7 ± 0.6	7.8 ± 0.5	25.0 ± 0.3	7.3 ± 0.3

Values are means of triplicate determinations. ND--not detected.

Table 2. Color removal efficiency of viable and nonviable Pseudomonas aeruginosa cells

	% Dye decolorization			
Dye used	Control (uninoculated)	Inoculated I (Killed cells)	Inoculated II (sodium azide treated)	Inoculated III (viable cells)
Basic violet 14	ND	3.1 ± 0.25	2.5 ± 0.15	78 ± 2.5
Basic violet 3	ND	3.4 ± 0.18	3.5 ± 0.28	70 ± 2.0
Acid blue 90	ND	4.5 ± 0.40	4.0 ± 0.37	54 ± 3.0

Values are means of triplicate determinations. ND--not detected.

3. Results and Discussion

3.1. Isolation of Dye-Degrading Bacteria

Five bacterial isolates that exhibited dye decolorization potentials on SWM agar spiked with basic violet 3 were picked and screened for their ability to degrade three triarylmethane dyes (basic violet 14, basic violet 3 and acid blue 90). The bacterial isolates decolorized the three dyes albeit to varying degrees (Table 1) within 24 h, and further decolorization of the dyes by the isolates was obtained after 36 h. The variation in decolorization efficiency of the isolates may be attributed to differences in the chemical structure of the dyes [18] and the varying metabolic functions of the different bacterial isolates. Decolorization of the dyes by the isolates was found to be due to degradation to a greater extent than adsorption as % adsorption obtained were quite low compared to % dye reduction by viable cells. Isolate ETL-1 did not show any evidence of adsorption after 24 h. Adsorption and/or degradation are the two mechanisms responsible for dye decolorization by microorganisms. Dye adsorption may be evident from inspection of the bacterial growth as those adsorbing dyes will be deeply colored, whereas those causing degradation will remain colorless. While the isolate ETL-1 cells cultured for 8 h with the dyes were colored, none of the cells was colored with any one of the dyes tested after incubation and decolorization for 24 h. Decolorization assay of a butanol extract of the cell pellets after incubation for 24 h with the dyes showed that the dyes were not adsorbed to the cell (data not shown). This indicates that decolorization of the dyes was mainly due to degradation rather than adsorption to cells. The deeply colored cell mats after 8 h may be explained by the fact that adsorption is frequently the first step of the biodegradation process before transportation of dye into the cytoplasm and its eventual breakdown by viable microbial cells. However, adsorption levels most times are an indication of biotransformation efficiency or its absence as rapid dye biodegraders rarely show high adsorption rates upon decolorization and incubation for an extended time period. In another experiment carried out using viable, autoclaved (killed), and metabolically poisoned cells of isolate ETL-1, dye decolorization was below 4.5% for the killed and poisoned cells while the viable cells exhibited color removal ranging from 54%-78% (Table 2). Hence, color removal exhibited by viable cells was attributed to the biotransformation of these dyes by the metabolic functions of the bacterium. Prior to now, various authors [28,29] had reported the isolation of a gene (*tmr*) encoding the enzyme, triphenylmethane reductase (TpmD), from bacteria, and we believe that this enzyme may be responsible for the bioconversion of the dyes tested. The visual change in biomass color of the killed and poisoned cells and their resuspension in methanol showed that the slight decolorization of their culture medium observed was due to adsorption of dyes on bacterial cells. Decolorization (adsorption) by dead cells may be due to the increase of the cell wall area that ruptured during autoclaving and may also be attributed to the revealing of special sites on cell walls [22]. Based on results, isolate ETL-1 which was identified as *Pseudomonas aeruginosa* was selected as having the best dye decolorization potential, since it showed little or no sorption of the three dyes and exhibited rapid decolorization of dyes within 24 h. ETL-1 was, thus, considered a good candidate for effective biological treatment of textile wastewater and used for further studies.

3.2. Sequence Analyses of Gene Encoding for the 16S rRNA from Bacterium ETL-1

The phylogenetic tree (Figure 7) constructed by the MEGA4 [24] displays ETL-1's position in relation to other members of the *Pseudomonas spp*., and 98% most closely related to *Pseudomonas otitidis* strain 81f and *Pseudomonas* spp GD6.

3.3. Effect of Agitation on Decolorization

The isolate, *Pseudomonas aeruginosa*, exhibited effective color removal activity only when incubated under shaking condition, whereas poor decolorization (< 30%) for the three dyes was obtained under static condition (Figure 1). Under agitated condition, decolorization percentages of dyes were 90%, 75%, and 66% for basic violet 14, basic violet 3, and acid blue 90, respectively, within 24h of incubation. Incubation under agitated condition was also necessary for better cell growth in contrast to incubation under static condition (data not shown). Poor decolorization of the dyes obtained under static condition could be attributed to the limitation of oxygen needed for the oxidative breakdown of the triarylmethane moiety, since enhanced decolorization was obtained when static cultures were subsequently incubated under agitated condition. *Pseudomonas aeruginosa* also exhibited maximum decolorization of dyes when 0.02% (w/v) yeast extract, starch or other carbon sources were supplemented in the medium (data not shown). In absence of a co-substrate, the bacterial culture showed reduced decolorization rates which suggested that the availability of a supplementary carbon source probably for generation of NADH molecules seems to be necessary for growth and decolorization of dyes. A previous report [46] had shown that both NADH/NADPH and molecular oxygen are necessary for the enzyme TpmD to decolorize triphenylmethane dyes which indicated that the enzyme is an NADH/NADPH-dependent oxygenase. Textile industrial wastewaters usually contain sizing agents such as starch, polyvinyl alcohol (PVA) and carboxymethyl cellulose added during sizing to provide strength to the fibers and minimize breakage [1], and these substances may serve as co-substrates for bacteria during effluent treatment for the generation of NADH molecules.

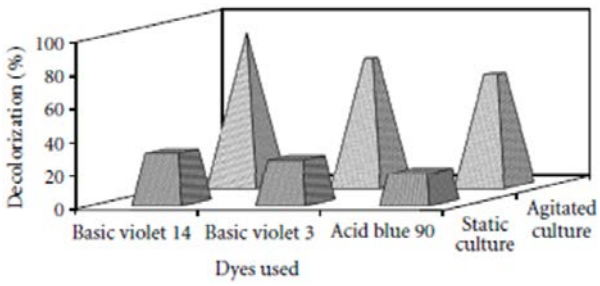

Figure 1. Decolorization of synthetic dye wastewater containing triarylmethane dyes under agitated and static conditions **Pseudomonas aeruginosa** (pH 7; 30°C; 24h). Values are means of triplicate determinations

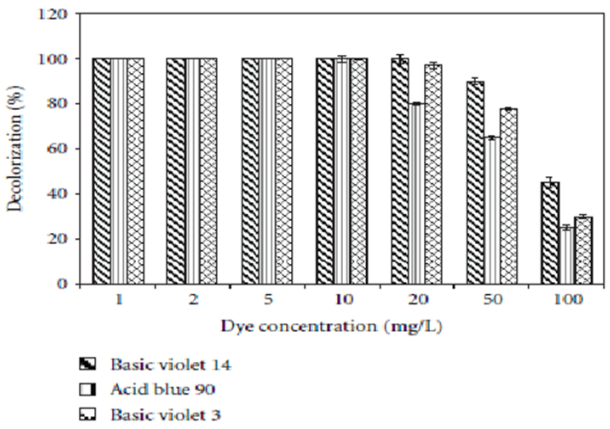

Figure 2. Decolorization of synthetic dye wastewater at different initial concentrations of triarylmenthanes dyes by **Pseudomonas aeruginosa** (pH 7; 30°C; 24h). Values are means of triplicate determinations. Error bars represent standard deviations

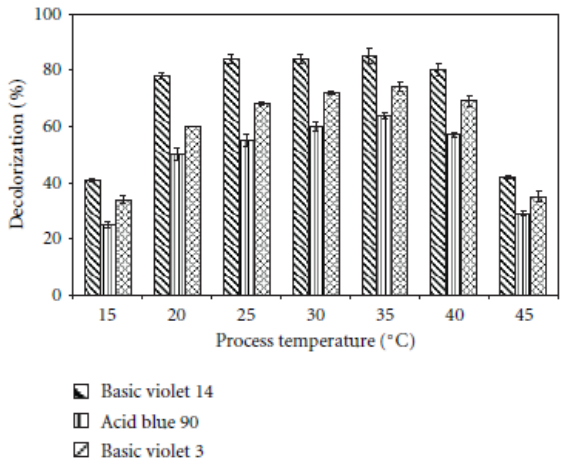

Figure 3. Decolorization of synthetic dye wastewater containing triarylmehae dyes at different incubation tempearatures by **Pseudomonas aeruginosa** (agitated culture; pH 7; 24h). Values are means of triplicate determinations. Error bars represent standard deviations

3.4. Effect of Initial Dye Concentration on Decolorization

The decolorization of dyes was studied at various increasing initial concentrations of each dye (1-100 mg/L). Results obtained show complete decolorization of dyes at initial concentrations between 1 and 20 mg/L within 24 h (Figure 2). However, decrease in % decolorization with increase in dye concentration was obtained at concentrations above 20 mg/L. Decolorization percentages of 90, 78, and 65 were obtained for basic violet 14, basic violet 3, and acid blue 90, respectively, at 50mg/l initial dye concentration, and this indicates that an acceptable high color removal can be achieved with *Pseudomonas aeruginosa* in culture broths with dye concentrations below 50mg/l. For industrial applications, it is important to know whether the microorganisms that decolorize dyes can bear high concentrations of the compound since the dye concentration in a typical industrial effluent can vary between 10 and 50mg/l [41]. *Pseudomonas aeruginosa* could decolorize the dyes at concentrations higher than those reported in waste waters and thus, it can be successfully exploited for treatment of dye bearing industrial waste waters. Zablocka-Godlewska et al. had reported that *Chryseomonas luteola* (42% removal) and *Pseudomonas aeruginosa* (40.5% removal) had the ability to decolorize 50 mg/l concentration of triphenylmethane dyes within 7 days. In comparison, our test isolate *Pseudomonas aeruginosa* showed 65%-90% decolorization of 50 mg/l of the triarylmethane dyes tested within 24h (Figure 2). These results show that *Pseudomonas aeruginosa* has a higher decolorization potential compared to the other bacteria reported previously. Decreased % decolorization of dyes obtained at higher concentrations suggests increasing dye toxicity with increase in dosage. Toxic effect was probably due to inhibition of cellular metabolic activities and cell growth. Several authors have also reported decreasing color removal with increasing dye concentration during decolorization of other dyes by bacteria [5,38,43].

3.5. Effects of Temperature and pH

In the experiments carried out at different temperatures, the initial dye concentration and pH were fixed at 50mg/l and 7 respectively, and the temperature effect was investigated at the range of 15°C-45°C. Results show that the temperature effect on decolorization was significant over the examined range (Figure 3) as dye decolorization increased as the temperature was elevated to 40°C. On further incubation, the same %dye decolorization was eventually reached in all flasks incubated at different temperatures suggesting the test isolate could acclimatize to a broad range of temperatures (15°C-40°C). The optimal temperature for bacterial activity was 35°C (Figure 3) and further increase in temperature beyond that resulted in marginal reduction in dye decolorization but essentially, thermal deactivation of decolorization activity under operational temperatures did not occur. Decline in bacterial activity at higher temperatures (>45°C) may be attributed to loss of cell viability or denaturation of the catabolic enzyme (38, 20). Determination of temperature requirements of microorganisms used for biotechnological applications is paramount, since temperature requirements above-ambient ranges may require an energy input and hence not cost effective. Effect of initial pH on the biodegradation efficiency of the isolate was analyzed over a pH range of 4 to 11 (Figure 4) To confirm if pH had any effect on decolorization of the dyes at the pH range studied, the absorbance signature of each dye was monitored at the different pH and no change in the visible spectra was obtained at this pH range. The degree of color removal observed varied with pH as increase in pH from 4 to 7 led to a threefold increase in % decolorization. Differences in biodegradation efficiency were insignificant between pH 7 and 9. Below pH 4 and above pH 10, negligible decolorization by this bacterium was observed. Though neutral pH 7 was best for degradation of the dyes, pH 9 appeared to be well tolerated and significant amount of dye could be degraded at that pH, thus indicating the potential of this organism to degrade dyes over a range of pH. Tolerance to varying pH by dye-decolorizing bacteria is quite important, as it makes them suitable for practical biotreatment of dyeing mill effluents [37,54]. However, to achieve the best rate of degradation, it is suggested that the pH of textile effluents be neutralized to 7. This trend of decolorization dependence on pH has been reported elsewhere [21,45]. They found that pH between 7 and 9 was optimum for decolorization of triphenylmethane dyes by *Pseudomonas otitidis* WL-13.

The effect of pH may be related to the transport of dye molecules across the cell membrane, which is considered a rate limiting step for dye decolorization [20]. At pH below 4, H^+ ions compete effectively with dye cations, causing a decrease in color removal efficiency, while at higher pH above this point charge, the surface of biomass gets negatively charged, which attracts the positively charged dye cations through electrostatic force of attraction [32].

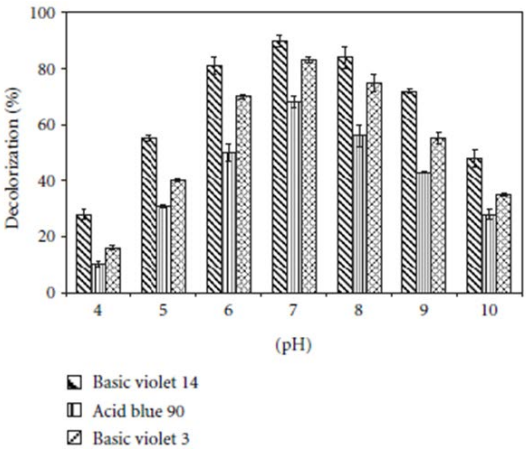

Figure 4. Decolorization of synthetic dye wastewater containing triarylmehae dyes at different initial Ph by **Pseudomonas aeruginosa** (agitated culture; 35°C; 24h). Values are means of triplicate determinations. Error bars represent standard deviations

3.6. UV-Visible Spectral Analysis

UV-visible spectral analysis was used to confirm that the decolorization process of the triarylmethane dyes was due to biodegradation. If dye removal is attributed to biodegradation, either the major visible light absorbance peak would completely disappear or a new peak will appear [22,50,51]. In adsorption examination, the absorption spectrum will reveal that all peaks decrease approximately in proportion to each other. Examination of spectral signatures of dyes indicated a decrease in the absorbance of samples withdrawn after decolorization using *Pseudomonas aeruginosa* (Figure 5). The absorbance peaks (at 0 h) of the dyes drastically reduced albeit at different rates within 24 h of incubation. No new absorbance peak appeared in samples after scanning following decolorization which indicates the breakdown of the dyes to non absorbing metabolites. The time courses of growth and dye decolorization by *Pseudomonas aeruginosa* when cultured in SWM containing triarylmethane dyes are presented in Figure 6. Increase in cell number was obtained with a concomitant decrease in dye concentration suggesting the utilization of the dyes by the bacterial cells for growth. Results obtained generally reveal color removal by the test isolate was largely due to the biodegradation of the dyes rather than adsorption. The optimal operational conditions for degradation of dyes by *Pseudomonas aeruginosa* were at pH 7-8 and incubation temperature of 35°C. Effective decolorization was obtained in medium containing initial dye concentration of 50mg/L or less. Similar results have been obtained as optimal conditions for degradation of azo and triphenylmethane dyes by other researchers [38,40,45]. In the case of Acid blue 90, the relatively higher molecular weight (854.02), its complex structure and the presence of sulphonic groups may be responsible for its lower biodegradability. Dyes with sulphonic groups are usually highly polar compounds [25], and this makes it difficult for them to penetrate into the cells through the cell membrane. The influence of dye structure on decolorization effectiveness had been demonstrated in a previous report [45]. The bacterial strain tested (*Citrobacter* sp.), removed faster and more effectively structurally simpler crystal violet and methyl red than more complicated gentian violet, malachite green and brilliant green. Similar dependence has been reported elsewhere [10,28]. In our study, *Pseudomonas aeruginosa* showed some advantages during dye decolorization such as robust growth property and simple growth requirements, which make it a potential strain for biotreatment of textile industrial effluent. PHA and PHBHHx are biopolymers which are accumulated by microorganisms as carbon and energy reserves [3,6,13]. These biopolymers have material properties similar to petrochemical plastics such as enhanced flexibility and improved impact strength, and yet, they are biodegradable and can be produced from carbon sources. The ability of *Pseudomonas aeruginosa* to produce PHA and PHBHHx during dye degradation should be explored as this may offer more advantages in the use of *Pseudomonas aeruginosa* as a biotechnological agent for generation of useful bioproducts during treatment of wastewater.

Table 3. Toxic effect of treated and nontreated synthetic dye wastewater on *Triticum aestivum*

Test parameters	Control	Basic violet 14		Basic violet 3		Acid blue 90	
		Untreated (50 mg/L)	Treated (50 mg/L)	Untreated (50 mg/L)	Treated (50 mg/L)	Untreated (50 mg/L)	Treated (50 mg/L)
Germination (%)	100^a	58^b	90^a	65^b	87^a	71^b	95^a
Shoot length (cm)	12.4 ± 1.3^a	7.5 ± 0.5^b	10.5 ± 0.7^a	7.0 ± 0.3^b	11.1 ± 0.5^a	8.3 ± 0.3^b	11.4 ± 0.4^a
Root length (cm)	10.8 ± 0.8^a	6.1 ± 0.2^b	9.2 ± 0.5^a	5.8 ± 1.1^b	9.6 ± 0.3^a	7.9 ± 0.7^b	9.5 ± 0.8^a
Seedling length (cm)	23.3 ± 1.2^a	13.8 ± 0.6^b	19.9 ± 0.8^a	12.6 ± 0.9^b	20.8 ± 0.5^a	16.4 ± 1.1^b	20.9 ± 0.7^a

Values are means of triplicate determinations, standard deviation (±), values followed by the same letter are not significantly different from the control (seeds germinated in SWM) at $P \leq 0.05$ according to the Dunett test.

Table 4. Toxic effect of treated and non treated synthetic dye wastewater on *Lens esculenta*

Test parameters	Control	Basic violet 14		Basic violet 3		Acid blue 90	
		Untreated (50 mg/L)	Treated (50 mg/L)	Untreated (50 mg/L)	Treated (50 mg/L)	Untreated (50 mg/L)	Treated (50 mg/L)
Germination (%)	100^a	50^b	90^a	40^b	75^a	62^b	95^a
Shoot length (cm)	13.6 ± 0.5^a	5.6 ± 0.7^b	10.7 ± 0.7^a	7.5 ± 0.3^b	12.0 ± 0.5^a	8.2 ± 0.3^b	13.0 ± 0.7^a
Root length (cm)	11.2 ± 0.2^a	5.7 ± 0.3^b	9.5 ± 0.6^a	6.5 ± 0.7^b	10.2 ± 0.6^a	7.2 ± 0.8^b	10.8 ± 1.2^a
Seedling length (cm)	24.9 ± 0.6^a	11.5 ± 0.5^b	20.2 ± 0.5^a	14.3 ± 1.1^b	22.6 ± 1.3^a	15.7 ± 0.8^b	23.7 ± 0.7^a

Values are means of triplicate determinations, standard deviation (±), values followed by the same letter are not significantly different from the control (seeds germinated in SWM) at $P \leq 0.05$ according to the Dunett test.

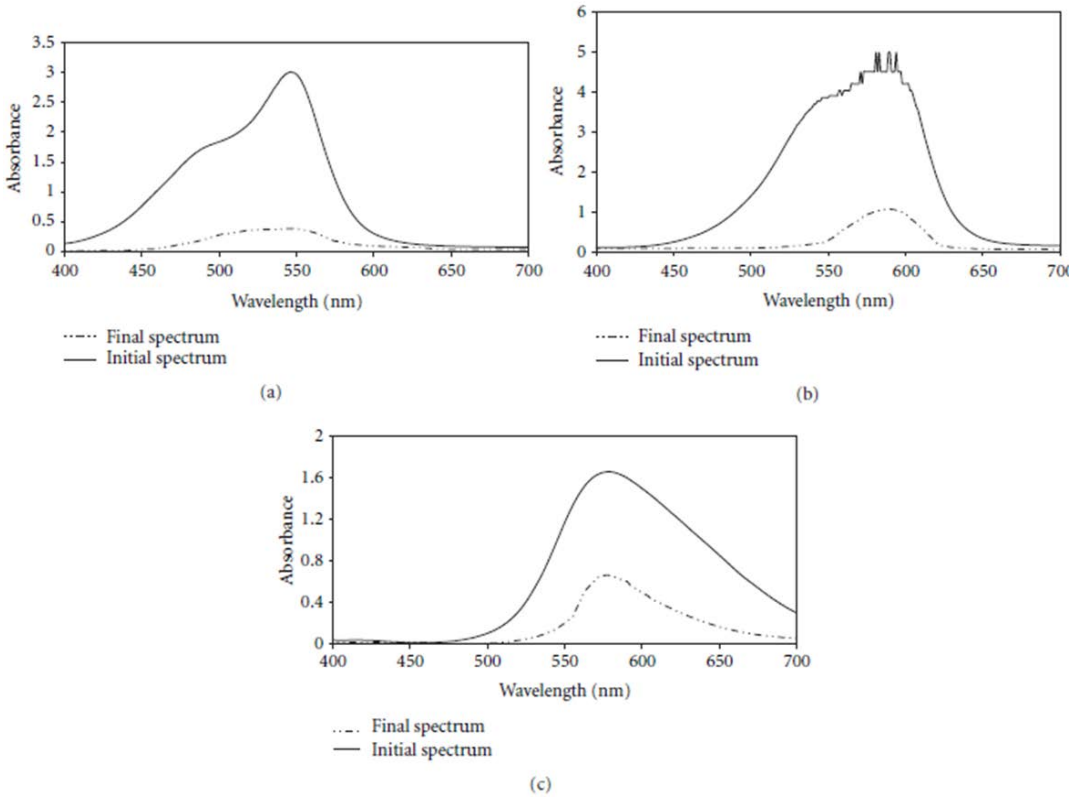

Figure 5. UV/visible spectra of triarylmethane dyes before and after degaradation by **Pseudomonas aeruginosa** in synthetic wastewater

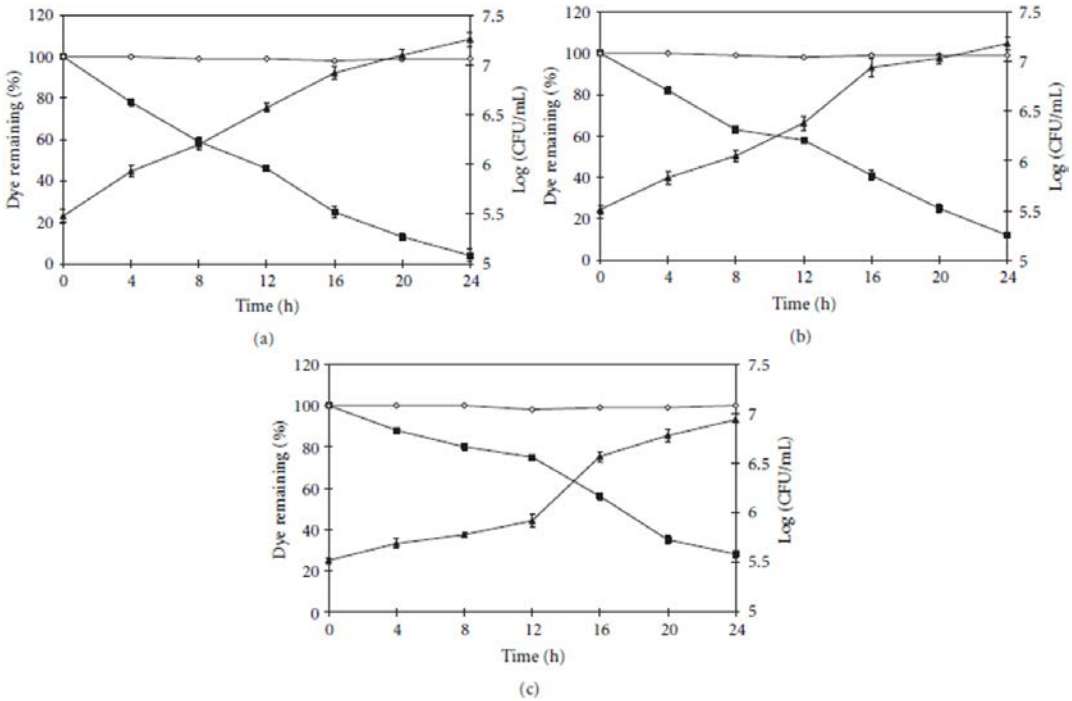

Figure 6. Time courses of growth and decolorization of triarylmethane dyes by **Pseudomonas aeruginosa** cultured in synthetic wastewater medium (50 mg/L initial dye concentration; pH 7; 35°C; 24h) under agitated condition. (a) Basic violet 14 (b) Basic violet 3 (c) Acid blue 0. (◊)—Control (uninoculated medium with dye); (■)—inoculated medium with dye; (▲)—Log cell number in culture. Data represent means (± SD) of triplicate experiments

3.7. Phytotoxicity Study

The disposal of untreated and treated textile dye wastewater on land may have a direct impact on soil fertility and by extension agricultural productivity. Thus, it was pertinent to assess the phytotoxicity of the dyes before and after degradation as environmental safety demands both pollutant removal and their detoxification. Phytotoxicity studies were carried out by evaluating the relative sensitivities of *Triticum aestivum*, and *Lens esculenta* toward the dyes and their degradation products using seed germination and plant growth assays. Germination (%) of plant seeds was less with the raw dye treatment when compared to the treatment with degradation metabolites and SWM. The lengths of shoot, root, and seedling were also significantly affected (Table 3,

and Table 4) by the three dyes than by their degradation metabolites, indicating less toxic nature of degradation metabolites as compared to dyes. Amongst the dyes, treatment with Basic violet 14 showed the most toxic effect on seed germination and plant growth parameters whereas, treatment with acid blue 90 exhibited the least toxic effect. However, for the dye metabolites, treatment with basic violet 3 showed the most toxic effect while Acid blue 90 exhibited the least toxic effect. Results indicate that effective dye decolorization does not always result in reduction of dye toxicity since basic violet 3 was better decolorized than acid blue 90. However, toxicity exerted by the treated samples was generally lower than that obtained for the untreated samples. Before now, most of the decolorization projects have concentrated mainly on color removal while neglecting the fact that sometimes biological processes are connected with formation of toxic intermediates. Hence, it is required that the evaluation of decolorization effect be carried out with relation to ecotoxicity assessment. In this study, phytotoxicity studies have revealed that the biodegradation of triarylmethane dyes by *Pseudomonas aeruginosa* resulted in their detoxification and generation of nontoxic metabolites thus suggesting biotreated dye wastewater can be used for irrigation.

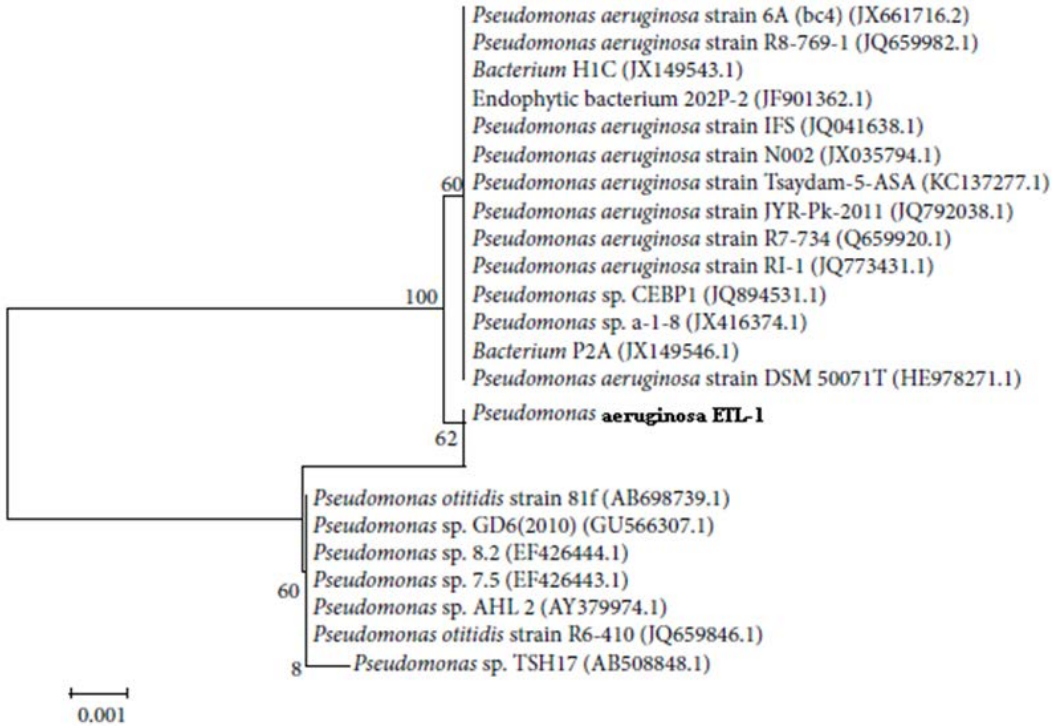

Figure 7. Phylogenetic tree based on 16S rRNA gene sequences, drawn using the neighbcur-joining methods, showing the relationships betweens strains ETL-1 and other *Pseudomonas* species

4. Conclusions

There are very few reports on the biodegradation and detoxification of textile and dye-stuff industrial wastes containing triarylmethane dyes. In this study, we describe the isolation and characterization of a strain of *Pseudomonas aeruginosa* capable of efficiently degrading triarylmethane dyes. The identity of the strain was confirmed using morphological, physiological, and biochemical assays. Degradation of dyes by the isolate was found to be dependent on dye concentration, aeration, pH, as well as temperature and presence of a cosubstrate. Phytotoxicity tests carried out on three plant species also indicated detoxification of the dyes after degradation as decolorized samples exhibited lower toxic effects than the raw dyes. Effective dye wastewater treatment using this isolate will demand the optimization of medium components and physicochemical conditions for maximum decolorization and detoxification. The advantages of this biological process are low cost, rapid degradation and simple handling and, hence, could be applied to treat wastewater from dyeing and printing operations and in bioremediation of dye contaminated environments. The next focus should be the design and scaling up of efficient tailor-made bioreactors with immobilized bacteria for the treatment of dye wastewater and to explore the potentials of producing useful biopolymer products from *Pseudomonas aeruginosa* during dye degradation.

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