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Research Article

Biodecolorization and Biodegradation of Azo Dye Reactive Orange-16 by Marine *Nocardiopsis* sp.

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Abstract

Background: Azo dyes are xenobiotic compounds that have bioaccumulated in the environment due to escalated industrial development. These are hazardous in nature, possessing carcinogenic and mutagenic effects on human beings.

Objectives: The perspective of the present study was to isolate and to determine azo dye (Reactive Orange-16) degrading potential of marine actinobacteria isolated from sediment samples of Port Blair, India.

Material and Methods: Actinobacteria with dye decolorization potential were isolated from sea sediment samples. The actinobacterial isolate with the highest dye decolorizing percentage was identified with the help of phenotypic, biochemical and molecular studies. The different physico-chemical parameters for dye decolorization were also optimized. The nature of decolorization by the potent isolate was determined with the help of High Performance Liquid chromatography (HPLC) and Fourier Transformed Infrared spectroscopy (FTIR) techniques. Further the toxicity of RO-16 decolorized products was investigated with the help of phytotoxcity assay.

Results: Out of six actinobacterial isolates, VITVAMB 1 possessed the most efficient RO-16 decolorization property. It decolorized 85.6% of RO-16 (250 mg L^{-1}) within 24hrs. Isolate VITVAMB 1 was identified to be *Nocardiopsis* sp. Maximum dye decolorization occurred at pH 8, temperature 35°C, 3% salt concentration and a dye concentration of 50 mg L^{-1}.

Conclusions: The nature of decolorization by *Nocardiopsis* sp. was biodegradation. Additionally, the degraded dye metabolites were found to be less toxic than pure dye. The high decolorization potential of VITVAMB 1 and the low toxicity of its degradation products make it a prospective dye removal system. The marine origin of VITVAMB 1 also makes it an attractive source for novel azo dye reducing enzymes.

Keywords: Biodegradation, Environmental; Spectroscopy, Fourier Transform Infrared; Chromatography, High Pressure Liquid; Reactive Orange-16

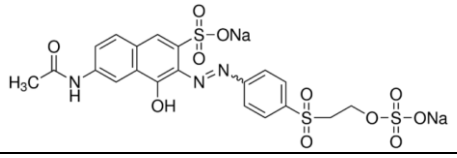
1. Background

Dye is a natural or synthetic substance used to add color. Its application in an aqueous solution must be followed by a mordant to increase the fastness of the dye on the fiber. Dye sticks to compatible surfaces by solution, by covalent bond formation or complex formation with salts or metals or by mechanical treatment (1). Such substances with substantial coloring ability find daily application in a wide range of industries including food, textile, pharmaceutical, cosmetics, photographic, and paper industries (2). Textile industries are an important contributor to the economic backbone of various nations. However, they are the major cause of environmental pollution such as land, air, water bodies and hazardous to living organisms. In the last decade, another element introduced for the environment friendliness of the finished merchandise, was the ban on certain azo dyes, which are known or suspected to be carcinogenic (3). More than 1,00,000 synthetic dyes exist so far and over 7×105 tons are commercially produced annually worldwide (2). Azo dyes comprise approximately 50% of the total dye production, and are the largest class among the synthetic dyes. They belong to the class of water soluble reactive dyes along with other dyes as anthraquinone, formazane and phthalocyanine. Azo dyes dominate the commercial market mainly because of the ease of their production

and the variety of colors they provide. In contrast to the organic pigments, color imparted by dyes are resistant to breakdown. The recalcitrant nature of the azo dyes are imparted by the presence of the azo group (N=N) which are not found in nature (4). The presence of azo group as a chromophore along with other auxochromes such as amine (NH2), carboxyl (COOH), sulfonate (SO3H) and hydroxyl (OH) is responsible for the vivid colors supplied by the azo dyes. Current knowledge about the long-term hazardous effects of dyes is still limited. Many disperse dyes in marine systems also show a connection to the bioaccumulation (5).

Different dyes are classified based on their chemical structure or chromophore. The classification of the Reactive Orange-16 (RO-16) dye used in this study is given in **Table 1**.

Table 1. The classification of the RO-16

Name	Chemical formula	Molar mass (g mol^{-1})	Structure	λ_{max} (nm)
RO-16	$C_2OH_{17}N_3Na_2O_{11}S_3$	617.54		492

RO-16 is commonly used in textile industries on a daily basis to dye cellulosic fibers and has poor fixation efficiency. A large amount of the dye is released along with the industrial effluents. Hence RO-16 was chosen for this study.

Due to low biodegradability of dyes, conventional effluent treatment systems are inefficient in treating industrial wastewater (6). The current physical or chemical treatment processes used includes coagulation, adsorption, oxidation, flocculation, filtration and electrochemical methods (7). Biological treatment of dye containing effluents offer an ecofriendly and low cost method to counteract pollution. There have been several reports of organisms which degrade dyes such as bacteria, fungi, yeast and actinomycetes (8). The biodecolorization of azo dyes involves either biodegradation (cleavage of azo bond) or biosorption (using living or dead biomass) process (9).

The marine ecosystem provides a unique and diverse range of actinomycetes. They are widely distributed in the intertidal zones, seawater, animals, plants, sponges, and also in ocean sediments. Marine environment is extremely different from terrestrial environment and is an important source of isolation of new species of actinomycetes producing novel bioactive compounds (10). Earlier, reports have been made on the degradation of hydrocarbons (11), explosives (12), chlorinated solvents (13), plasticizers (14) and azo dyes (15) by terrestrial actinomycetes. However, marine actinomycetes were less explored for their ability in biodegradation of hazardous dyestuff. Hence, in this study marine actinobacteria have been utilized for the biodecolorization and degradation of azo dyes.

2. Objectives

The aim of this study was to explore the marine environment for the isolation of actinobacteria capable of decolorizing azo dye Reactive orange-16 and also to determine the nature of decolorization.

3. Materials and Methods

3.1. Chemicals

Azo dye was obtained from a textile industry in Mysore, Karnataka, India (12.2958° N, 76.6394° E). The dye used in this study was C.I. RO-16.

Starch casein agar (SCA), glucose, peptone, manganese chloride ($MnCl_2.4H_2O$), ethyl acetate, potassium bromide (KBr) were purchased from HiMedia Laboratories Pvt. Ltd., India. Soluble starch, yeast extract, beef extract, glycerol, calcium carbonate ($CaCO_3$), zinc sulphate ($ZnSO_4$), copper sulphate ($CuSO_4.5H_2O$), ferrous sulphate ($FeSO_4.7H_2O$), sodium nitrate ($NaNO_3$) and mercuric chloride ($HgCl_2$) were obtained from Sisco Research Laboratories Pvt. Ltd., India. HPLC grade carbinol was procured from Thomas Baker (chemicals) Pvt. Ltd, India. All the chemicals used to carry out these experiments were of highest purity.

3.2. Sample Collection

Marine sediment samples were collected from Port Blair, India 11°40′06″N 92°44′16″E. Samples were collected in sterilized containers, and plastic bags. For further use, it was transferred to the laboratory and stored at a temperature of 4°C.

3.3. Isolation and Screening of Actinobacteria

Sediment sample was serially diluted and 0.1 µL aliquots were plated on SCA plates. Inoculated plates were incubated at room temperature for a period of 7-10 days. Isolates with distinct colony morphology were selected and subcultured repeatedly on SCA plates to obtain pure cultures (16).

Primary screening of the isolates was performed in Soluble Starch broth (composed of starch 25 g, glucose 10 g, yeast extract 2 g, trace salt solution 1 mL, $CaCO_3$ 3 g, distilled water 500 mL and sea water 500 mL) as well as fermentation broth (consisting of glycerol 70 mL, glucose 30 g, beef extract 30 g, peptone 80 g, NaNO3 20 g, and 1 L of sea water,

maintained at pH 7) for 5-7 days at room temperature. Broth cultures were then observed for decolorization (17).

3.4. Decolorization Assay of Actinobacterial Isolates Against RO-16

Decolorization of the broth cultures were analyzed by UV-Vis Spectrophotometer (Hitachi U-2800) at an absorbance maximum (λ_{max}) of 492 nm for RO-16. Actinobacterial isolates were cultured in fermentation media consisting of RO-16 at a concentration of 250 mg L^{-1}. Culture broths were then incubated at 28°C for a period of 7 days. After incubation, culture broths were centrifuged at 6,708 ×g for 15 min. Supernatant was collected and analyzed spectrophotometrically for dye decolorization at regular time intervals. Negative control and blank was maintained. The extent of dye decolorization was quantified using the following formula (8):

$$\%\text{Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

3.5. Optimization of Dye Decolorization Process

Some of the physico-chemical factors which might have an effect on dye decolorization process were investigated. The maximum decolorization percentage was monitored by varying the pH (pH 4, 5, 6, 7, 8, 9, 10), temperature (25°C, 35°C, 45°C, 55°C), NaCl concentration (1%, 2%, 3%, 4%, 5%) and dye concentration (50 mg L^{-1}, 100 mg L^{-1}, 150 mg L^{-1}, 200 mg L^{-1}, 250 mg L^{-1}, and 300 mg L^{-1}).

3.6. Identification of the Potential Dye Degrading Actinobacteria

The potential isolate VITVAMB 1 with high dye decolorizing ability was chosen for identification. Phenotypic characteristics (aerial mass color, mycelial morphology, gram character, acid-fastness) were observed and biochemical tests (catalase, oxidase, nitrate reduction, citrate utilization, starch hydrolysis, gelatin liquefaction, melanin production, decomposition of xanthine, hypoxanthine, esculine, urea, and carbohydrate fermentation tests) were performed. Phenotypic and biochemical characterization was performed as described by Shirling & Gottileb (1966), and Bergey's manual of determinative bacteriology (18). Further molecular studies (16S rRNA gene sequencing) was done to identify the isolate to the species level. The 16S rRNA gene was isolated and amplified. The resulting gene sequence was then aligned with closely related sequences from NCBI using MUSCLE software program. Further, a phylogenetic tree was constructed using the software MEGA 5.

3.7. Analysis of Degraded Metabolites by Fourier Transform-Infrared spectroscopy (FT-IR), and High Pressure Liquid Chromatography (HPLC)

The degraded metabolites obtained after dye degradation were extracted by solvent extraction method using ethyl acetate. These extracted metabolites were then dried in a rotary evaporator. The dried metabolites thus obtained were used for further analysis. The dried sample was used directly for FT-IR analysis. FT-IR was carried out in mid IR range of 400-4000 cm^{-1} at a scan speed of 16. The dried metabolite was uniformly mixed with spectroscopically pure KBr in a ratio of 1:9, the mixture was fastened firmly in sample holder, and then analyzed.

HPLC analysis of the ethyl acetate extracted sample and that of the pure dye was carried out (Waters model no. 1525) with UV-detector set at 492 nm. The stationary phase was a C_{18} column (4.6×250 mm) and the mobile phase was HPLC grade carbinol/water (55:45 v/v). The flow rate was 0.8 mL min^{-1} and the run time was 20 min.

3.8. Phytotoxicity Study

The phytotoxicity assay was performed using seeds of *Vigna radiata* (moong dal) in order to investigate the toxicity of pure dye (RO-16) and its degraded metabolites. The concentration of RO-16 and its degraded metabolites used for this study was 100 ppm. Seeds treated with distilled water was used as control. The seeds were first surface sterilized using 1% HgCl2 solution and then cleansed with distilled water for further use. Seeds were then kept on wet cotton placed in a petridish and regularly treated with a fixed volume of dye, degraded metabolite and control was maintained with distilled water for a period of 7 days. After 7 days percentage of the germinated seeds was calculated from each set. The shoot length of the germinated seeds was also measured (19).

3.9. Statistical Analysis

In this study were performed in triplicates. Decolorization assay and optimization studies were statistically analyzed using one way as well as two way ANOVA. The results were established based on probabilities and significance was considered at 95% confidence level.

4. Results

4.1. Isolation and Screening of Actinobacteria for Decolorization of RO-16

A total of six actinobacterial isolates (VITVAMB 1, VITVAMB 2, VITVAMB 3, VITVAMB 4, VITVAMB 5, VITVAMB 6) were recovered from the marine sediment samples. Among them only three isolates showed the dye decolorizing potential (VITVAMB 1, VITVAMB 4 and VITVAMB 6) during primary screening. Further decolorization assay was performed using VITVAMB 1, VITVAMB 4 and VITVAMB 6.

4.2. Decolorization Assay

The actinobacterial isolate VITVAMB 1 showed 85.6% of decolorization of RO-16 within 24 h. Whereas, VITVAMB 6 and VITVAMB 4 showed a decolorization of 52% and 42.34 % of RO-16 with 24 h respectively. The isolate VITVAMB 1 showed rapid increase in percentage of decolorization after 12 h and the percentage increased with time elapsed. Comparatively, VITVAMB 4 and VITVAMB 6 showed a gradual increase in decolorization (**Fig. 1**).

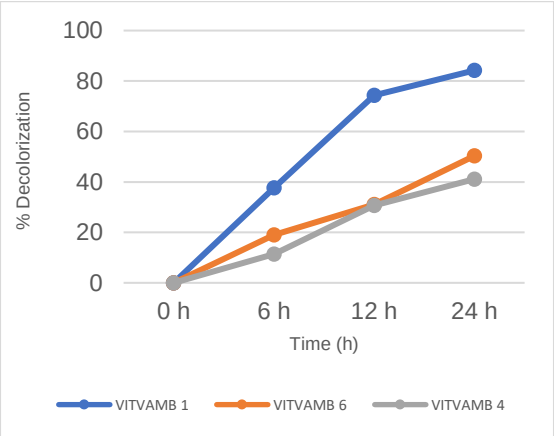


Figure 1. Decolorization percentage of RO-16 by VITVAMB 1, VITVAMB 6 and VITVAMB 4 at specific time intervals

4.3. Optimization of Dye Decolorization

The varying physico-chemical parameters had a considerable impact on the dye decolorization percentage. The effect of different pH showed highest decolorization of 94.5% at pH 8 followed by 86% at pH 7. Decolorization was greatly reduced at acidic pH range as well as alkaline pH range (**Fig. 2a**). When temperature was varied, maximum decolorization of 94.13% occurred at 35°C. Isolate VITVAMB 1 was capable of maintaining a good decolorization potential within the temperature range of 25-45°C. However decolorization was reduced to almost half on increasing the temperature to 55°C (**Fig. 2b**). Salt concentration of 3% produced the highest decolorization of 94.62%. Comparable decolorization (90.42%) occurred at 2% salt concentration. Dye decolorization was greatly reduced at higher salt concentrations of 4% and 5% (**Fig. 2c**). The effect of varying dye concentration was also studied and good dye decolorization was observed till 250 mg L^{-1}. Decolorization gradually decreased with increasing dye concentration and highest decolorization (95.073%) occurred at the lowest dye concentration (50 mg L^{-1}). Dye decolorization was lowered to 56.39% at 300 mg L^{-1} (**Fig. 2d**).

4.4. Identification of the Potential Dye Degrading Actinobacteria

The potential isolate VITVAMB 1 was identified to belong to the genus *Nocardiopsis* when subjected to phenotypic and biochemical characterization. The aerial mass color was white and was abundant, fragmenting and branched in morphology. The substrate mycelium was long and densely branched, fragmented into coccoid shape. It was gram positive and non- acid fast bacteria. It showed positive reaction for oxidase, catalase, starch hydrolysis, nitrate reduction, citrate utilization and gelatine liquefaction. It was capable of decomposing xanthine, hypoxanthine and esculine, but did not decompose urea. Melanin production was not identified. Among the sugars, VITVAMB 1 utilized glucose, rhamnose, maltose, fructose, galactose, starch, but was incapable of fermenting sucrose, arabinose, inositol, sorbitol.

Based on 16S rRNA molecular studies VITVAMB 1 was identified to be *Nocardiopsis dassonvillei* sp. Basic local alignment search tool (BLAST) was used to analyze the consensus sequence of VITVAMB 1. Strain VITVAMB 1 had close phylogenetic affiliation to the genus *Nocardiopsis*. The phylogenetic tree formed shows that strain VITVAMB 1 has 100% similarity with *Nocardiopsis dassonvillei* subsp. *Albirubida* strain NBRC 13392 (**Fig. 3**). The 16S rRNA gene sequence of VITVAMB 1 is available in the NCBI database with the accession number MF616329.1.

4.5. FT-IR Analysis

The FTIR analysis of both the pure dye RO-16 and decolorized dye product were carried out to identify and compare the different functional groups present in both the compounds. Spectrum of the pure dye showed –OH group at 3265.49 cm^{-1}, while 29339.73 and 2879.72 cm^{-1} represented C-H stretch respectively. C-N stretch was observed at 1035.77 cm^{-1}, 993 cm^{-1} corresponded to C=H out of frame vibration and 850 cm^{-1} for S-OR ester. Signature azo group (N=N) of the dye was observed at 1637.56 cm^{-1} (**Fig. 4a**).

On the contrary, FT-IR spectrum of the decolorized dye product showed changes in the position of the peak formation. There was a shift of OH group to 3300.20 cm^{-1}, and the azo group to 1635.654 cm^{-1} (**Fig. 4b**). The absence of all other peaks shows the distortion of the compound, thus it can be concluded that the dye structure has been degraded.

4.6. HPLC Analysis

The HPLC chromatogram of the pure dye RO-16 at the beginning of static incubation has been shown in **Figure 5(a)**. The chromatogram shows a sharp major peak with a retention time of 1.756 min. After decolorization occurred, the chromatogram of the degraded dye metabolite showed a considerable decrease of the major peak. After degradation a major peak was formed at 2.552 min and two other peaks were formed at 2 min and 3.5 min respectively (**Fig. 5b**). Disappearance of the major peak of the pure dye

RO-16 and appearance of new peaks at different retention time in the HPLC chromatogram of degraded dye metabolite indicates degradation of the dye.

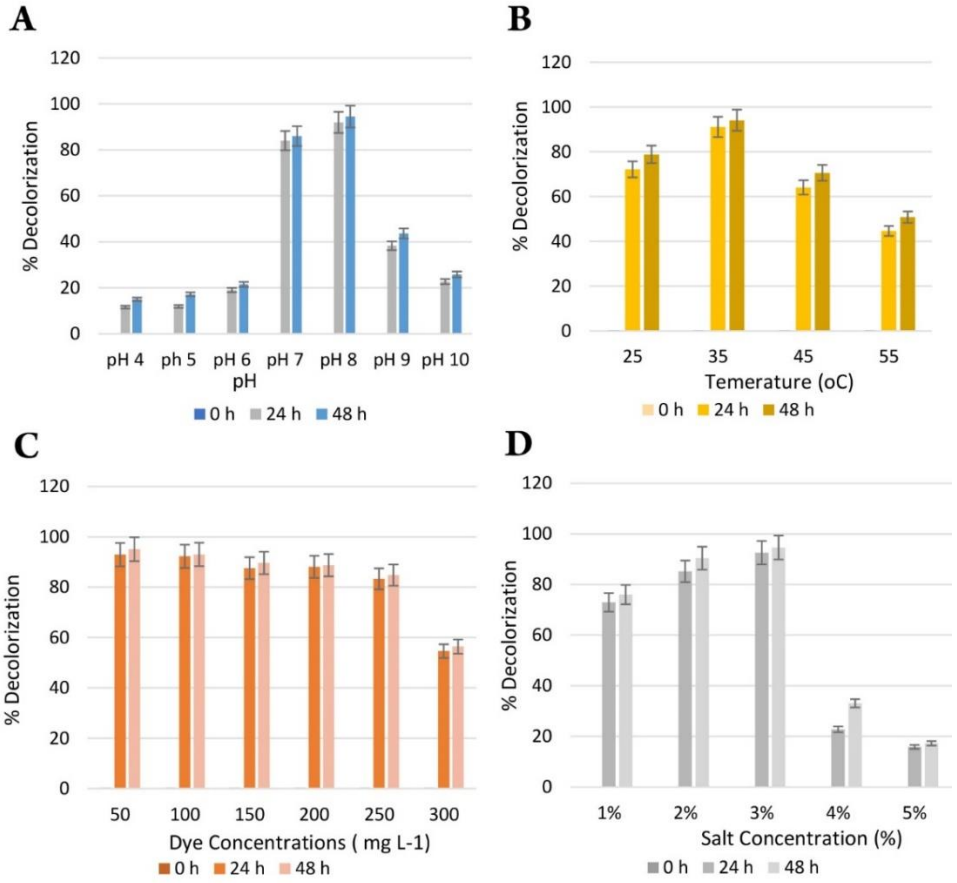


Figure 2. Optimization of Dye decolorization under different physico-chemical parameters (a) pH; (b) temperature; (c) salt concentration; (d) dye concentration

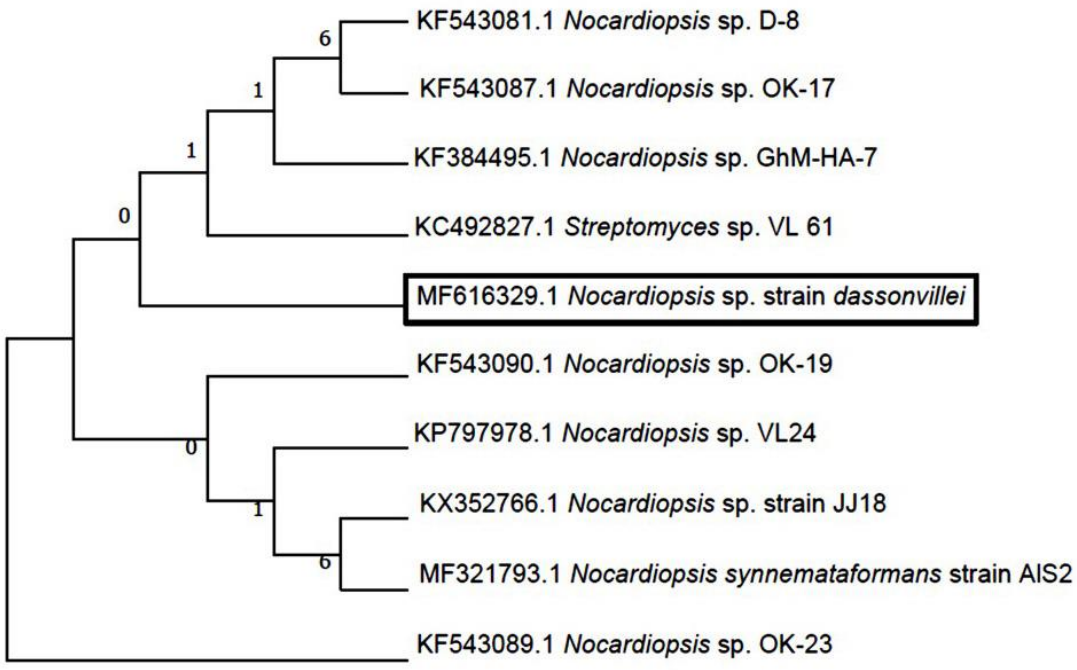


Figure 3. Phylogenetic Tree of Isolate VITVAMB 1

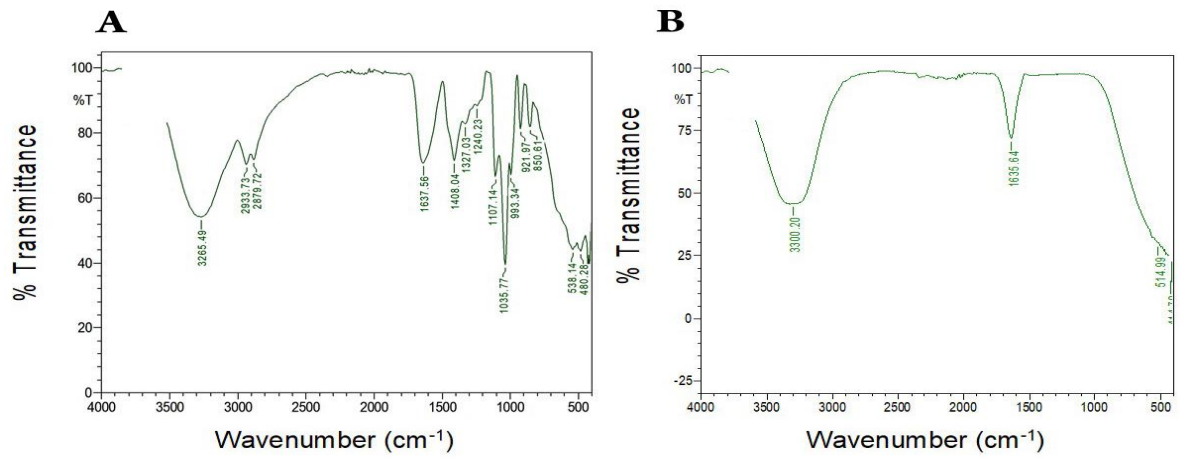


Figure 4. FT-IR Analysis A) Spectrum of Pure Dye RO-16; B) Spectrum of Degraded RO-16 Metabolite by VITVAMB 1

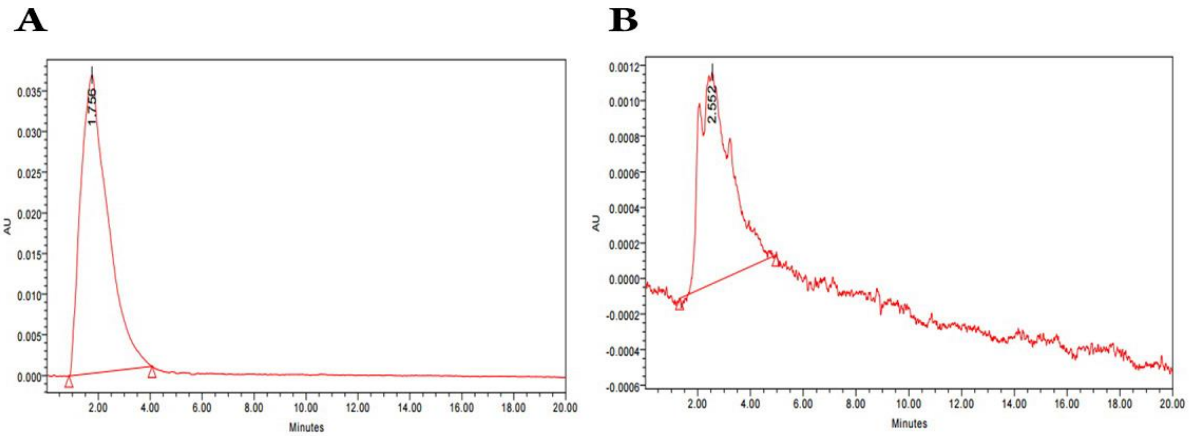


Figure 5. HPLC Chromatogram A) Pure Dye RO-16; B) Degraded Dye Metabolite of RO-16

4.7. Phytotoxicity Study

Phytotoxicity assay results to determine the toxicity of pure dye RO-16 and degraded dye produtcs conducted with seeds of *Vigna radiata*. Seeds which underwent treatment with degraded dye metabolites (100 ppm) and control treatment showed a germination rate of 50% and 95% respectively, whereas seeds treated with RO-16 (100 ppm) showed a very low germination rate of only 10%. Similar trend was observed for shoot length analysis. Seeds treated with RO-16 had very less shoot length of about 0.13 ± 0.035 cm, while seeds treated with degraded dye metabolite showed an intermediate shoot length of 0.8 ± 0.153 cm, which were on par with the control seeds with a shoot length of 1.1 ± 0.231 cm.

Values represented in the assay were mean of three experiments ± SEM, and were found to be significantly different from the control treatment at $P < 0.05$, by one way analysis of variance (ANOVA).

5. Discussion

Microbial clean-up of toxic pollutants in the environment is a foremost and important area of research owing to the rising levels of environmental pollution. Azo dyes are the major commercial dyes used in a wide variety of industries and are toxic xenobiotic compounds. Their recalcitrant nature resists their biodegradation. In the present study, actinobacterial isolates from the marine environment were studied to examine their potential to decolorize and degrade azo dye RO-16. A total of six marine actinobacterial isolates were obtained and three of the isolates (VITVAMB 1, VITVAMB 6 and VITVAMB 4) showed dye decolorization ability. In a similar study, actinomycetes were obtained from marine sediments and three isolates belonging to the genera of *Micromonospora* sp., *Streptomyces* sp. and *Micropolyspora* sp. showed the ability to decolorize Amido Black (20). Another study reported the isolation of 15 different strains of actinomycetes, out of which *Nocardia* sp. demonstrated dye decolorizing ability of Congo Red (21). In the present study the isolate VITVAMB 1 showed a decolorizing potential of 85.6% at a dye concentration of 250 mg L-1 (RO-16) within 24 h and was identified to be Nocardiopsis sp. strain dassonvillei. Previously, Shobana and Thangam (22) reported the decolorizing ability of *Nocardiopsis alba* isolated from acclimated sludge and dying waste water. *N. alba* was able to decolorize 83-85% of the dye RO-16 at a concentration of 250 mg L-1 which is on par with the current study. In

another study, a novel marine actinomycetes strain *Streptomyces* sp. VITDDK3 showed 98% decolorization of azo dye Reactive Red 5B at a concentration of 50 mg L^{-1} (23). Although decolorization was higher in this case, but the concentration of dye used was much less than that of the present study. Several researches proved that physico-chemical parameters as pH, temperature, salinity, cations, anions, biological oxygen demand, chemical oxygen demand have profound effect on degradation of various environmental pollutants (19, 24). In this study the consequence of varying pH, temperature, salt concentration and dye concentrations on dye decolorization were studied. Highest decolorization occurred at a slightly alkaline pH of 8. In an earlier report, Streptomyces krainskii SUK-5 showed complete decolorization of Reactive Blue-59 at pH 8 (25). Previous studies have also proved that actinobacterial enzymes show optimal activity at pH 8 (26, 27). Industrial processes utilizing reactive dyes operate at alkaline pH. Thus the ability of VITVAMB 1 to show maximum decolorization at an alkaline pH can be beneficial for industrial application. Temperature plays crucial role in dye degradation as observed in previous studies (28-30). In the present study maximum decolorization occurred at 35°C. Similar studies reported that *Streptomyces* sp. showed optimal dye degradation at a temperature of 40°C (30). *N. alba* reportedly showed maximum decolorization of RO-16 at a temperature of 30°C (22). Textile wastewater often consists of heavy amounts of inorganic salts especially chlorides and sulfates (31). In this research, the effects of varying concentrations of sodium chloride on dye decolorization was studied. Dye decolorization showed a gradual increase of decolorization with increasing salt concentration. Highest decolorization (94.62%) occurred at 3% salt concentration. However, higher salt concentrations of 4% and 5% showed a sudden decrease in decolorization potential. Similar results were reported by (19). Dye concentration was also varied and dye decolorization gradually decreased with increased dye concentration. Highest decolorization (95.07%) occurred at lowest concentration (50 mg L^{-1}). Isolate VITVAMB 1 showed good decolorization potential till a dye concentration of 250 mg L^{-1}. Dye decolorization diminished to 56.39% at 300 mg L^{-1}. The decrease in decolorization ability with escalating dye concentration can be ascribed to be the toxic effects of pure dye imposed on the microbial cells. Similar effect was observed in actinomycete Georgenia sp. CC-NMPT-T3. Maximum decolorization of 94.2% occurred at the lowest concentration (50 mg L^{-1}) and decolorization decreased to 85.24% at 500 mg L^{-1} (32). Further, the mechanism of dye decolorization was biodegradation, which was evident from the results of FT-IR, and HPLC analysis. FTIR spectra of the extracted metabolite of dye degradation showed absence of major functional groups which were present in the spectra of the pure dye. This indicates that the dye molecule was degraded. HPLC chromatogram of the degraded dye metabolite showed displacement of the major peak and formation of two other peaks at different retention times when compared to that of the pure dye RO-16. Several reports have been made earlier, which used HPLC and FT-IR to identify dye degradation. In 2009, Saratale *et al.* showed the biodegradation of Scarlet R by a bacterial consortium using FT-IR and HPLC (33). Similarly, Kalyani *et al.* (2009) used FT-IR and HPLC to identify the degradation of the dye Reactive Red 2 by *Pseudomonas* sp. SUK1 (34). It is essential to prove that the degraded dye metabolites so produced are non-toxic for its successful field application. Phytotoxicity assay provides certain advantages as it is economical, takes less time, has similar chromosomal morphology to mammals, and produces similar response to mutagens. Numerous phytotoxicity assays had been conducted earlier with seeds of plants such as *Sorghum vulgare*, *Phaseolus mungo* and *Vigna radiata* to determine the non-toxic nature of the degraded dye metabolites (35-37). Here, *V. radiata* seeds were used to conduct phytotoxicity assay. Assay results revealed that the pure dye affected the germination rate of the seeds when compared to the control, while the degraded dye metabolite showed less germination rate than control but more compared to the pure dye. Similarly, shoot length was also measured and found to be highly affected by the pure dye but not with degraded dye metabolite, when compared to control.

6. Conclusions

Numerous researches have been carried out to counteract the dye removal problem. However, there still lies a dearth of an effective treatment system to address the issue of dye pollution. Marine environment has been known from a long time to be largely unexplored and harbors organisms producing novel secondary metabolites. Hence in this study, marine environment was used as a source for isolating azo dye decolorizing actinobacteria. Isolate VITVAMB 1 identified as *Nocardiopsis* sp. *dassonvillei* showed high decolorization potential of RO-16 at 85.6% at a concentration of 250 mg L^{-1}.

The optimum pH, temperature, salt concentration and dye concentration were investigated. Maximum decolorization occurred at pH 8, temperature 35°C, 3% salt concentration and 50 mg L^{-1} dye concentration. Isolate VITVAMB 1 decolorized RO-16 by biodegradation and the biodegraded metabolites were non-toxic in nature. So far, no reports have been made on the decolorization of RO-16 by marine actinobacterium *Nocardiopsis dassonvillei*. The hostile marine environment might be responsible for producing novel secondary metabolite in the isolate VITVAMB 1 which was responsible for dye decolorization and biodegradation. Further, the nontoxic nature of the degraded products makes them highly potential to be applied for human welfare.

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