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Research

DECOLOURISATION OF DYES AND ITS MIXTURE BY *PROVIDENCIA* SP. VNB7 ISOLATED FROM TEXTILE EFFLUENT TREATMENT PLANT

NIRANJAN PRAKASHRAO PATIL [1]*, JUMMA SHAIKH [1], B.P. KAPADNIS [3] AND V.B. GAIKWAD [2]

[1] Department of Microbiology, Abasaheb Garware College, Savitribai Phule Pune University, Pune, Maharashtra, India

[2] Department of Environmental Sciences, KTHM College, Nashik, Maharashtra, India

[3] Department of Microbiology, Savitribai Phule Pune University, Pune, Maharashtra, India

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ABSTRACT

To address coloured textile effluent treatment problems, eco-friendly method demands versatile microorganism exhibiting repertoire of attributes. This study focuses on isolating bacteria from textile effluent having standalone potential to decolorize and degrade efficiently mixture of dyes. The isolate named *Providencia* sp. VNB7 isolated, identified and evaluated for decolourisation of 11 structurally different dyes and mixture of 10 of them (DM10). The decolourisation of 500ppm DM10 achieved was 79% after 12 h and 95% after 24 h of incubation. The optimum condition for decolourisation of DM10 with isolate *Providencia* sp VNB7 were viz. static/anoxic incubation, pH 7, 35°C temperature, 1.5% (w/v) NaCl and 150 ppm DM10 concentration. The degradation of DM10 and Malachite green was monitored using UV-visible spectroscopy. The induction of laccase, lignin peroxidases, tyrosinases (oxidative) and azo reductase (reductive) enzyme activities in presence of DM10 confirmed degradation of dyes. Hence, we suggest isolate *Providencia* sp. VNB7 to be promising contender for decolourisation of coloured textile effluent.

INTRODUCTION

The dyes are not the absolute needs but aesthetic needs of mankind. Azo dyes are synthetic dyes extensively used in industries. Due to inefficiencies in dying process, approximately 10% to 15% of the dyes are released in to environment (Jadhav, *et al.*, 2008). It was estimated that 280,000 tons of textile dyes find its way in to the effluent (Jin, 2007; Saratale, 2011). The demand and use of dyes is continuously increasing putting pressure on environment. Many dyes are visible in water at concentrations as low as 1 ppm (Banat, 1996). The discharge of untreated colored textile effluent affect penetration of sunlight in large water bodies (lake, river) disturbing balance of aquatic flora and fauna in the ecosystem (Vandevivere, *et al.*, 1998). Several dyes and their metabolites are carcinogenic, mutagenic and toxic to life forms (Myslak, *et al.*, 1998). This necessitates suitable treatment of dyes containing effluent in to nontoxic form before discharge. The azo dyes are most commonly used dyes and are resistant to chemical and biological degradation due to their complex chemical structure and synthetic origin. Several physicochemical methods have been applied for the removal of dyes from wastewater effluent. However, physical/chemical methods have inherent drawbacks over eco-friendly microbial or enzymatic decolourisation (Vandevivere, 1998; Robinson, 2001; dos Santos A, 2007; Saratale, 2011). The research on bacteria mediated decolourisation processes is most frequently pursued and extensive work on role of pure culture and consortia in decolourisation of dyes under aerobic (Wong, 1998; Adedayo, 2004; Kodam, 2005; dos Santos, 2007;), anaerobic (Moutaouakkil, 2003; Pearce, 2006) and microaerophilic conditions exist (Hong, 2007; Xu, 2007; Kalme, 2007; Franciscon, 2012). Recently, a bacterium *Providencia* sp was isolated and employed for decolourisation and degradation of Acid black 210 (Jadhav, *et al.*, 2008) and

***Corresponding author's email:** niranjan75@gmail.com

Dispersed red 78 (Agrawal, *et al.*, 2014) separately. Very few study reported decolourisation of mixture of dyes. A consortium of *Rhodococcus globerulus*, *Proteus mirabilis* and *Aeromonas caviae*, was evaluated against 16 azo dyes individually and also a mixture (Lade, *et al.*, 2015). The objective of present research work is to isolate and identify bacterial species from textile effluent and evaluate its performance *in vitro* with respect to decolourisation of mixture of textile dyes. We report individual 11 dyes and DM10 decolourisation capabilities of isolate *Providencia* sp. VNB7 in pure culture.

MATERIAL AND METHODS

Dyes, chemical and media

The dyes used in this study were purchased from M/s. Oswal dyechem (Mumbai, India). The chemical used were reagent grade. The microbiological media and components were purchased from HiMedia (India). The stock solutions of dyes (without purification) were prepared by dissolving 0.5 g of powder in 100 ml of distilled water autoclaved after mixing. The DM10 was prepared by adding all the dyes in equimolar concentrations. The λ_{max} of each dye was determined in nutrient broth using spectrophotometer.

Isolation and characterization of isolate

The samples were collected from the different stages of effluent treatment (aeration tank, equalization tank and centrifuge decanter) plant of the Naroda Common Effluent treatment plant, Ahmedabad which receives effluent from the approximately 250 dye manufacturing and utilizing industries around. The sample was enriched in test tube of sterile nutrient broth containing 200 ppm DM10 under static condition. The test tube showing visible dye decolourisation was selected and used to carry out streak plating on nutrient agar containing 200 ppm DM10. The colony surrounded by zone of clearance was transferred on nutrient agar slant. The isolates were evaluated for their decolourisation ability in nutrient broth containing dye mixture. The promising bacterial isolate having greatest decolourisation potential was characterized using routine microbial identification test (morphology, cultural and biochemical) in our laboratory. The isolate was further characterized by automated Vitek® 2 System version 03.01 (BioMerieux) and partial sequencing of 16S rRNA gene at molecular diagnostic center (Pune, India). The 16S rRNA gene sequence of the isolate was submitted to the GeneBank.

Decolourisation experiments

The *Providencia* sp. was grown in nutrient broth for 18 h under shaking condition. 5% inoculum of 1 OD at 600 nm was used to inoculate nutrient broth containing 50 ppm dye. The decolourisation experiment was carried out under static/anoxic condition at 35°C. After regular interval the sample were drawn and centrifuge at 10000 rpm for 15 min. The absorbance of supernatant was read at λ_{max} of respective dye using spectrophotometer (Shimadzu, 1700 model). %D is the dye decolourisation calculated as (Xu, 2007; Kalme, 2007)

$$\%D = \frac{(\text{Initial Absorbance} - \text{Final Absorbance})}{\text{Initial absorbance}} \times 100$$

The % Decolourisation of DM10 was reported as the average decolourization of λ_{max} of constituent dyes.

Effect of physicochemical factors

For optimization experiments, 50 ppm of DM10 (10 dyes) in nutrient broth was used to study effect of shaking (120 rpm) and static condition. The dye malachite green was not added in the mixture of dyes as it precipitated dye mixture. The decolourisation of DM10 was studied with respect to varying dye concentrations (50 ppm to 250 ppm), temperature (20°C to 50°C), pH (3 to 11) and NaCl concentration (0 g% to 10 g%).

UV-vis spectroscopy of dyes

The decolourisation of dyes and dye mix was monitored using UV-vis spectroscopy. The decolourisation experiment was set up and aliquots were removed after definite time interval. The centrifugation of sample was carried out at 15000 rpm for 15 min. The spectral scan (range 200 nm to 800 nm) of supernatant of decolorized dye broth at various time intervals was compared with un-inoculated control broth.

Oxidative and reductive enzymes of DM10 decolourisation

The investigation of various enzymes involved in bioremediation process of dyes was done by measuring comparative enzyme activities in dye induced broth and un-induced control (without dye). The cell free extract of cells of *Providencia* sp. was prepared according to the method given by Agrawal, *et al*. The Laccase enzyme was assayed using Guaicol (Joshi, *et al.*, 2008), Lignin peroxidases (Bains, *et al.*, 2003) using n-propanol, azoreductase (Shanmugam, *et al.*, 1999) using methyl red and tyrosinases (Chen, *et al.*, 2005) using catechol as substrate.

Statistical analysis

The data was analyzed by t-test. The observations

Table 1. Details of dyes and their decolorization by *Providencia* sp. VNB7 after 12 h of incubation

Sr.	Dye	C.I. No.	Class	λ_{max} (nm)	% Decolorization*
1	Reactive Red 195	31221002	Diazo sulphone	540	77 ± 1.61
2	Reactive Black 5	20505	Vinyl sulphone diazo	598	75 ± 1.48
3	Golden Yellow145	31221018	Monoazo sulphone	420	96 ± 1.83
4	Chrysophenine G	24895	Diazo sulphone	403	87 ± 1.87
5	Acid Orange II	15510	Vinylsulphone monoazo	483	78 ± 1.34
6	Methyl Red	13020	Acid monoazo	430	98 ± 0.37
7	Acid black 210	300285	Vinyl sulphone triazo	604	89 ± 1.13
8	Methyl orange	13025	Acid sulphone monoazo	465	98 ± 0.52
9	Indigo carmine	73015	Indigoid	586	83 ± 1.42
10	Amaranth	16185	Monoazo sulphone	520	85 ± 1.6
11	Malachite Green	42000	Triphenyl methane	617	89 ± 1.6
11	DM10	-	-	-	77 ± 2.13
*values are represented as arithmetic mean ± standard deviation					

were considered significant when P was ≤ 0.05. The means ± standard deviations or SEM were calculated from three replicates.

RESULTS AND DISCUSSION

Isolation and characterization of isolate

The criteria used for selecting potential isolate were time and specificity of decolourisation towards structurally different dyes. The screening program yielded isolate VNB7 showing promising results in liquid medium containing various dyes and DM10. The colorless cell pellet was obtained after centrifugation of dye broth. The decolourisation of dyes and DM10 was initiated from the bottom of tube. In order to know taxonomic identity of isolate VNB7, morphological, biochemical and 16S rDNA analysis was performed. The bacterium was Gram negative, short rods, motile, catalase positive and oxidase negative. The commercial biochemical identification tests (bioMérieux')) for Gram negative (VITEK®2 GN ID) bacteria predicted 95% probability for *Providencia alcalifaciens*. The 16S rRNA gene sequence BLASTN search shown 100% identity with *Providencia alcalifaciens* strains DSM 30120 (accession number NZ_ABXW01000071). The sequence was deposited to GenBank sequence database under accession number GQ478243. In our opinion the combined results of phenetic characters, biochemical characters and 16S rRNA gene sequence similarity may be sufficient for assuming isolate VNB7 taxonomic identity as *Providencia alcalifaciens*.

Table 1 illustrates decolourisation capability of isolate against a range of dyes. The isolate decolorized 11 different dyes and DM10. The decolourisation range was between 74% to 98% within 12 h for different dyes. The lowest % decolourisation was obtained for dye and highest decolourisation for methyl red and methyl orange (98%). Based on decolourisation of dyes studied relationship cannot be established with respect to dye structure and decolourisation efficiency. Methyl red, methyl orange, golden yellow, acid black 210, reactive red 195 and acid orange 2 were completely decolorized (visible) by *Providencia* sp. after extended incubation time. As products of decolorized dyes also contribute absorbance at the λ_{max} of dyes, hence technically absorbance cannot diminish to zero. The isolate exhibited important attribute of decolourisation of DM10, offering suitability in treating blend of dyes. (Lade, *et al*., 2014) reported 98% decolourisation of azo dye dispersed red 78 by *Providencia rettgeri* strain HSL1 within 36h., while (Agrawal, *et al*., 2013) reported >99% decolourisation of triazo Acid Black 210 by *Providencia* sp. SRS82 within 24 h. Our results extend potential of *Providencia* sp. in decolourisation of various textile dyes.

Effect of physicochemical factors on decolourisation

To know the combination physicochemical factors influencing decolourisation process, optimization was performed. Practically the effluent contains mixture of various dyes hence DM10 was chosen over single dye as it is closest to the real time scenario. The decolourisation (Fig. 1) in aerobically grown flask (18%) was affected drastically compare to the decolourisation under static condition (95%). In aerobic condition, there is competition between oxygen and azo dyes as terminal electron acceptor. The presence of oxygen favors the growth and inhibits the azo reduction of dyes, dominating utilization of NADH in aerobic respiration (Zhang, *et al.*, 1997). The pellet in aerobically grown system was colored due to biosorption of dyes on cells which lead to 33% decolourisation of DM10 i.e. from liquid phase to solid. Table 2 summarizes various optimized factors in relation to their ranges in which >75% decolourisation was achieved. Based on data from table we can conclude that *Providencia* sp. decolorized DM10 in reasonably broad range

of parameters offering flexibility dynamics of manipulation in treatment process.

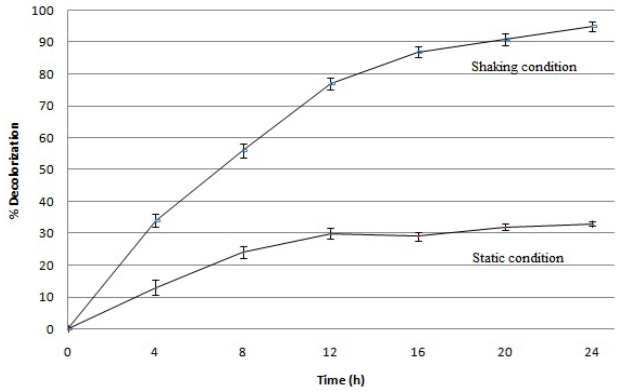

Fig. 1 Effect of oxygen on decolorization of DM10 by *Providencia* sp. VNB7.

Table 2. Optimization of DM10 decolorization using *Providencia* sp. VNB7.

Factors	Range investigated	Operational range*	Optimized factor
[DM10] (mg/L)	50-500	50-250	150**
pH	3-10	6-8.5	7.0
Temperature (°C)	20-50	25-45	35
NaCl (g%)	0-10	0.5% to 6%	1.5
Oxygen relationship	Static and agitated	-	Static
* >75% decolorization of 50 ppm DM10, ** highest decolorization rate obtained			

Monitoring decolourisation

Fig. 2 shows UV-vis spectrum of DM10 decolourisation at different reaction time. The UV-vis spectral pattern of treated and untreated DM10 is markedly different. The hypochromic shift (lower absorbance) from wavelength approximately 375 nm to 700 nm and appearance of new peak at approximately wavelength 260 nm indicate azo reduction or degradation of dye molecules. Fig. 3 illustrate the decolourisation of Malachite green by isolate as it is not the constituent of DM10. The significant differences in control and treated malachite green imply Triphenyl reductase activity. The absorption peak at 605 nm and 425 nm wavelength was diminished and hyperchromic shift occur between 200 nm to 300 nm wavelengths. The primary aromatic amines produced after azo reduction of dyes have absorption maxima in this range. These results suggest that dye removal by *Providencia* sp. might be principally attributed to biodegradation

Enzymes involved in decolourisation of DM10

The analysis of UV-vis results prompted us to detect and quantify activities of enzymes performing degradation of dyes in DM10. Table 3 show various oxidative (laccase, lignin peroxidases and tyrosinase) and reductive (azo reductase) enzymes activities. The activities of enzymes were significantly induced in presence of DM10 suggesting their involvement in decolourisation and degradation of DM10. Similar enzymes activities were detected and reported by *Providencia* sp. in scientific literature (Jadhav, 2008; Agrawal, 2014).

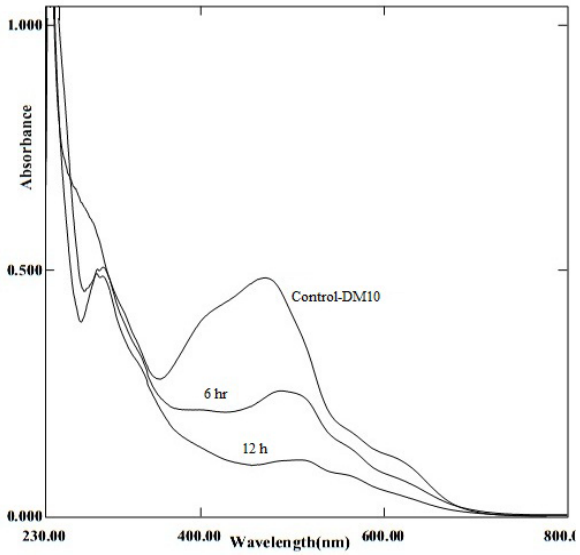

Fig. 2 UV-Vis spectral changes during decolorization of DM10 by *Providencia* sp. VNB7.

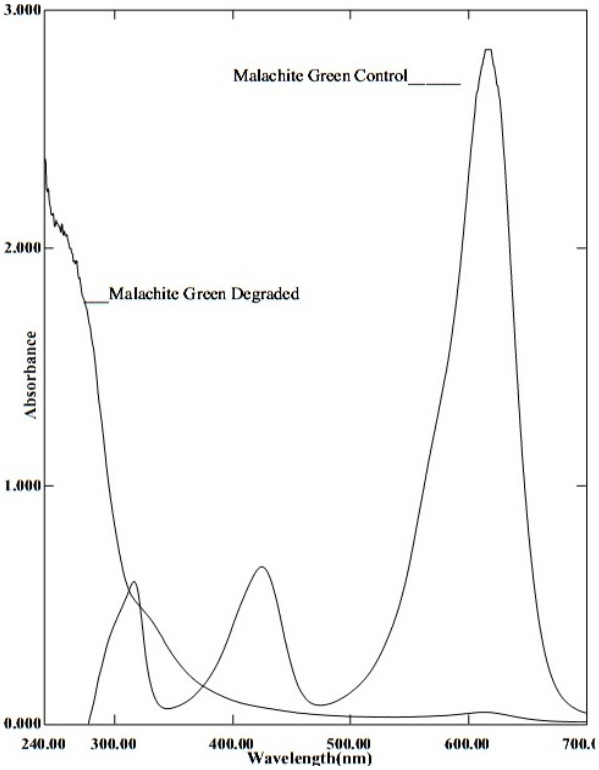

Fig. 3 UV-Vis spectral changes during decolorization of malachite green by *Providencia* sp. VNB7.

Table 3. Effect of DM10 on enzyme activities of *Providencia* sp.VNB after 12 hrs of incubation

Enzymes	Enzyme activity# Without DM10	Enzyme activity#(Induced) with DM10
Azoreductase[a]	0.102 ± 0.008	0.163* ± 0.012
Lignin peroxidase[b]	0.089 ± 0.012	0.187* ± 0.010
Laccase[b]	0.103 ± 0.011	0.289* ± 0.006
Tyrosinase[b]	0.058 ± 0.005	0.084* ± 0.009

values are mean of three experiments (±) SEM, Significantly different from the without DM10 (Un-induced) at *P<0.05, significant by paired t-test. [a] µg Methyl red reduced min^{-1}mg $protein^{-1}$. [b] $Umin^{-1}$mg $protein^{-1}$

CONCLUSION

The bacterium *Providencia* sp. isolated from dye polluted industrial effluent has ability to degrade 11 structurally different dyes and mixture of 10 of them. The broad dye decolourisation activity of isolate is beneficial to claim commercial application. The decolourisation was performed at moderate physicochemical conditions. The collective result of UV-Vis spectroscopy studies and oxidative and reductive enzymes involved in decolourisation process confirm the degradation of dyes. We opine that; isolate *Providencia* sp. VNB1 exhibit applicability in suitable dye decolourisation process.

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