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Decolorization of Methyl Red by Staphylococcus saprophyticus strain AUCASVE3 Isolated from Textile Effluent

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Abstract

In this study, an attempt was made to isolate methyl red, an azo dye, decolorizing bacteria from textile effluent. Among three isolates with methyl red decolorizing ability, the potential bacterial isolates, *Staphylococcus saprophyticus* strain AUCASVE3 was selected for identification and characterization. The organism was found to belong to the genus *Staphylococcus* and species *saprophyticus*. The isolate *Staphylococcus saprophyticus* strain AUCASVE3 could decolorize 600, 700, 800, and 1000 mg/L of methyl red within 24 h and 48 h, respectively, in presence of 1.0 g/L of glucose. This bacterium showed 94 and 97% methyl red (600 mg/L) decolorization within 24 and 48 h, respectively. In case of temperature, the ranging between 30-40ºC was fond to be suitable for the decolorization by *S. saprophyticus* strain AUCASVE3 and a further increase in the temperature drastically affected decolorization activity of bacterial culture. Thus, due to high potential for methyl red decolorization the isolate *Staphylococcus saprophyticus* strain AUCASVE3 can be used in the biological treatment plant of industrial effluent containing azo dyes.

Keywords: decolorization; methyl red; 16S rRNA genes; *Staphylococcus saprophyticus* strain AUCASVE3

1. Introduction

Dyes are the colored substances which fixes firmly with the substrate. Synthetic dyes are used extensively for textile dying, paper printing, and color photography. Approximately 10,000 different dyes and pigments are used industrially and over 7 X 10^5 tons of these dyes are produced annually worldwide. Dyes are broadly classified in to several types. Based on the chemical structure of chromophoric group, synthetic dyes are classified as azo dyes, anthraquinone dyes, triarylmethane dyes, etc. These dyes have an adverse effect on the environment. Disposal of effluent in soil and river prevents the penetration of sunlight in them. These Dyes are toxic, carcinogenic and genotoxic because of their high COD values. In textile industries azo dyes play a major role in colouring process. Azo dyes are considered as electron deficient xenobiotic components because they possess azo (N=N) and| sulphonic (-SO_3-) electron withdrawing groups, generating electron deficiency and making the component less susceptible to oxidative catabolism by bacteria. They are not typically degraded under aerobic conditions. Azo dyes are mainly used in dyeing textile fibers, particularly cotton but also silk, wool, viscose and synthetic fibers. At present there are around 3,000 azo dyes in use worldwide and they account for 65% of the commercial dyes. Azo dyes are the largest group of dyes used in industry (Ramalho *et al*., 2002) representing more than half of the annual production (Stolz, 2001). It has been estimated that about 10% of the dye stuff used during this dyeing processes does not bind to the fibers and is therefore released into the sewage treatment systems or environment (Zollinger, 1987).

Microbial decolorization and degradation is an alternative approaches to physical and chemical degradation processes of color removal, which is environment friendly and cost effective (Wesenberg *et al*., 2003). Most studies on azo dye biodegradation have focused on bacteria and fungi, which are able to biodegrade and bioadsorb the dyes in textile industry effluents (Pearce *et al*., 2003; Eichlerova *et al*., 2006). The organisms used in most of the studies were *Staphylococcus* sp, Escherichia coli, Bacillus sp, *Clostridium* sp, and *Pseudomonas* sp. in bacteria (McMullan *et al*., 2001). Mechanism for decolorization of azo dyes by microrganims has been proposed by Keck *et al*. (1997). Anaerobic and micro-aerophilic microorganisms reduce azo bonds non-specifically in anaerobic conditions leading to dye decolorization.

In the present study, the potentiality of the aerobic decolorization of methyl red (MR) by *Staphylococcus saprophyticus* strain AUCASVE3 growing on a synthetic medium was studied. This bacterial strain, isolated from dye contaminated sludge, decolorizes

methyl red. The effects of different temperatures on the decolorization of methyl red by *Staphylococcus saprophyticus* strain AUCASVE3 were studied and compared. Objectives of present work are as follows:

1. Isolation, screening and identification of highly Potential azo dye (methyl red) decolorizing bacteria.
2. Determination of color removal efficiency of methyl red by isolated bacteria.

2. Materials and Methods

2.1. Isolation of methyl red decolorizing bacteria

Dye-contaminated sludge was collected from a wastewater outlet of an industrial area of Dhaka Export Processing Zone (Saver).

Approximately, 1 g of sludge was suspended in 10 ml of sterile 0.85% sodium chloride solution (w/v) and mixed thoroughly. The mixture was serially diluted with sterile 0.85% sodium chloride solution. Aliquots of 0.1 ml of 10^{-1}, 10^{-2} and 10^{-3} dilutions were spreaded onto minimum medium (MM) plates containing: K_2HPO_4, 1.36 g/L; $MgSO_4$, 0.1 g/L; $(NH_4)_2SO_4$, 0.6 g/L; $CaCl_2$, 0.02 g/L; NaCl, 0.5 g/L; $MnSO_4$, 1.1 mg/L; $ZnSO_4$, 0.2 mg/L; $CuSO_4$, 0.2 mg/L; $FeSO_4$ 0.14 mg/L; MR, 100 mg/L; agar, 15 g/L and pH was adjusted to 7 with HCl and NaOH (Moutaouakkil *et. al*., 2003) All plates were incubated at 37 °C for 3 days. Colonies surrounded by decolorized zones were picked and streaked onto MM plates containing 100 mg/L of MR. The plates were again incubated under the same conditions to confirm their abilities to decolorize MR.

2.2. Preparation of methyl red solution

Methyl red was dissolved in 50 per cent ethanol. Stock of 1000 mg/L methyl red solution was prepared by dissolving 10.0 g of methyl red in 1000 ml 50 per cent ethanol. The methyl red solutions for the experiments were prepared by diluting the stock solution to obtain desired concentrations.

2.3. Test of decolorizing potentialities by using Staphylococcus saprophyticus strain AUCASVE3 organism

For this test viz. methyl red at different concentrations were used. This dye compound at different concentration viz. 600, 700, 800, and 1000 µg/ml was mixed with mineral salt media. These modified broths were inoculated with the *Staphylococcus saprophyticus* strain AUCASVE3 isolated from the waste samples and incubated at 37ºC for 7 days. The decolorization was observed periodically.

2.4. Spectrophotometric analysis of the dye tested

Percentages of dye decolorization by the culture were measured by UV-visible spectrophotometer (Jenway 6310). Decolorization of the methyl red was determined at the respective maximum absorption wavelength (430 nm) in the culture supernatants using spectrophotometer. Samples were aseptically taken into the centrifuge tube at different times intervals during incubation. Cells were removed by centrifugation at 5000 rpm for 10 minutes. Percentage of dye decolorization was calculated by the following formula.

2.5. Identification of the bacterial isolate

$$\% \text{ of decolorization} = \frac{\text{Sample concentration}}{\text{Standard concentration}} \times 100$$

Bacterial staining, cultural, morphological and biochemical procedures were studied for the identification of bacterial isolate. Isolated bacteria were identified according to methods described in Bergey's Manual of Determinative Bacteriology (Buchanan, 1984).

2.6. Sequence based identification of isolates

The isolates can be identified based on alignment of partial sequence of 16S rRNA gene with the existing sequences available in the database. In the present experiment the bacterial isolates were used to amplify their 16S rRNA gene. PCR amplified DNA of the isolate was gel purified using phenol freeze method and sent for automated sequencing (Applied Biosystem 3130). The sequence generated from automated sequencing of PCR amplified DNA was analyzed through NCBI BLAST (http://blast.ncbi.nlm.nih.gov/) program to find out possible similar organism through alignment of homologous sequences.

3. Results and Discussion

3.1. Isolation and screening of methyl red decolorizing bacteria

Methyl red decolorizing bacteria were isolated from textile effluent. On the basis of their decolorizing capacity, a total of three bacteria were isolated and purified by repeated sub-culturing. These three bacteria had potentials to grow on liquid culture media

containing 400 to 800 mg/L of methyl red and decolorized it. These three isolates were designated as TX1, TX2 and TA3.

3.2. Identification of bacterial isolate

Preliminary identification test were performed for isolates TX1, TX3 and TA1. Other identification techniques including morphological characteristics, biochemical test were also performed. Sequence analyses of 16S ribosomal RNA (16S rRNA) were performed of the isolates by amplifying the 16S rRNA genes by PCR using the bacterial universal primers 27f and 1492r. The PCR products purified through alcohol precipitation was sequenced directly using a DNA auto sequencer (Applied Biosystem 3130). The most closely related sequence was found using the BLAST programs. Morphological characteristics, colony characteristics and other biochemical characteristics of these isolates are outlined in Table 1. Bioinformatics of these strains able to grow well on and use azo-dye as carbon source are shown in Table 2.

In order to identify the bacterial isolates, their 16S rRNA genes were amplified and sequenced. Strain TA-1 was affiliated to *Bacillus megaterium* strain H2 (99% similarity), strain TX-1 to *Staphylococcus saprophyticus* strain AUCASVE3 (99%), and strain TX-3 to *Staphylococcus saprophyticus* strain A20 (92%) (Table 2).

Table 1. Morphological and biochemical characteristics of the isolates

Morphological and Biochemical Characteristics	**Strain No.**		
Morphological Characteristics	**TA-1**	**TX-1**	**TX-3**
Form	Circular	Circular	Circular
Elevation	Effuse	raised	raised
Margin	Entire	Entire	Entire
Surface	Smooth	Smooth	Smooth
Color	White	Off White	Slightly yellow
Shape and arrangement of cells	Rods, rounded end, occur in chain	Cocci, occur in singly, and tetrads	Cocci, occur in singly, and tetrads
Motility	-	-	-
Biochemical Charactertics			
Gram reaction	+	+	+
Oxidase test	-	-	-
Catalase test	+	+	+
Oxygen requirement	Facultative anaerobes	Facultative anaerobes	Facultative anaerobes
Starch hydrolysis	+	-	-
Gelatin liquefaction	+	+	+
VP test	-	+	+
MR test	-	+	+
Deamination of Phenylalanine	-	-	-
Utilization of Citrate	-	-	+
Acid production from D- Glucose	+	+	+
Gas production from D- Glucose	-	+	+
Name of the isolates	*Bacillus megaterium*	*Staphylococcus saprophyticus*	*Staphylococcus saprophyticus*

Table 2. Bioinformatics of the strains able to grow well on and use azo-dye as carbon source

Name of the isolates	Name and Accession number of the Bacterial strain having highest similarity		Basis of the bioinformatics parameters					
	Accession Number	Strain Name	Score	Bits	Query coverage	Expect value	Identities	Gaps
TX-1	JQ043188.1	*Staphylococcus saprophyticus* strain AUCASVE3	1000	541	89%	0.0	99%	2/551 (0%)
TX-3	HQ323432.1	*Staphylococcus saprophyticus* strain A20	451	244	55%	2e-123	92%	10/333 (3%)
TA-1	JQ579631.1	*Bacillus megaterium* strain H2	1002	542	90%	0.0	99%	3/562 (1%)

The 16s rRNA gene sequencing of the *Staphylococcus saprophyticus* strain AUCASVE3

***Staphylococcus saprophyticus* strain AUCASVE3**

GCTTATATTGACTGGCATCTCTCCAGGCGGAGTGCTTATGCGTTAGCTGCAGCACTAAGGGGCGGAAA
CCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCA
CGCTTTCGCACATCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTC
TGCGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCAATGA
CCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTACGCGCGCTTTACGCCCA
ATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTC
TGATTAGGTACCGTCAAGACGTGCACAGTTACTTACACGTTTGTTCTTCCCTAATAACAGAGTTTTACG
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CTGCTGCCTCCCGAGGGAATTCTGGA

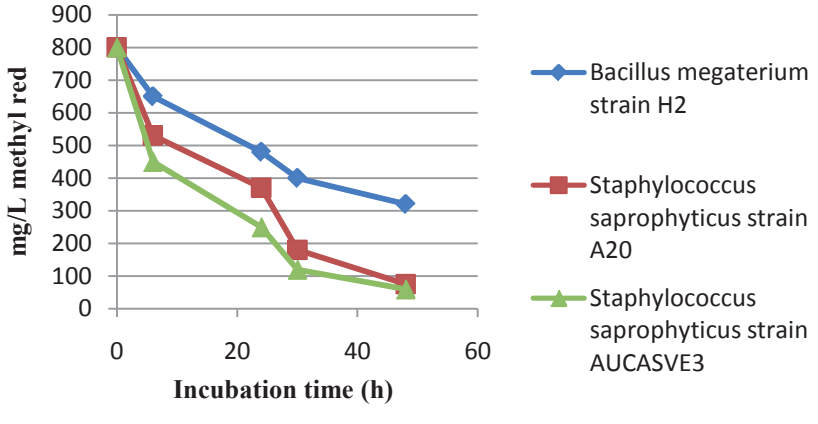
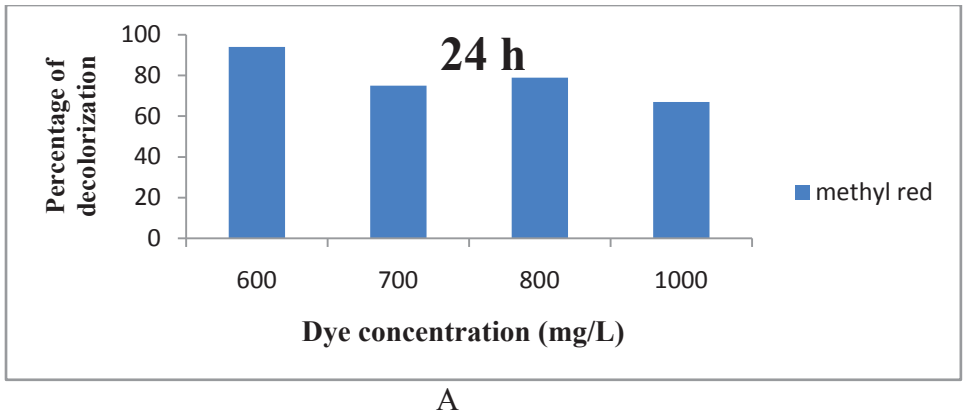
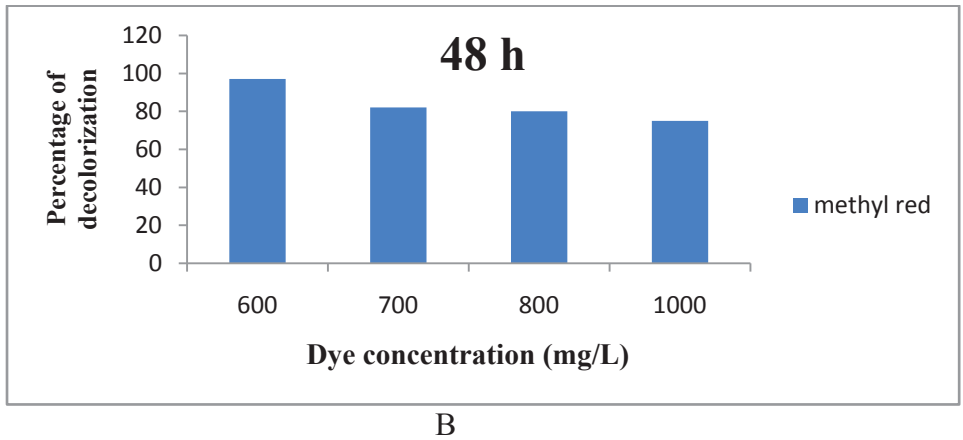


Figure 1. Decolorization of 800 mg/l methyl red by isolates TX-1 (*Staphylococcus saprophyticus* strain AUCASVE3), TX-3 (*Staphylococcus saprophyticus* strain A20) and TA-1 (*Bacillus megaterium* strain H2).



A



B

Figure 2. Percentage of decolorization of various concentrations of methyl red by the isolate *Staphylococcus saprophyticus* strain AUCASVE3.

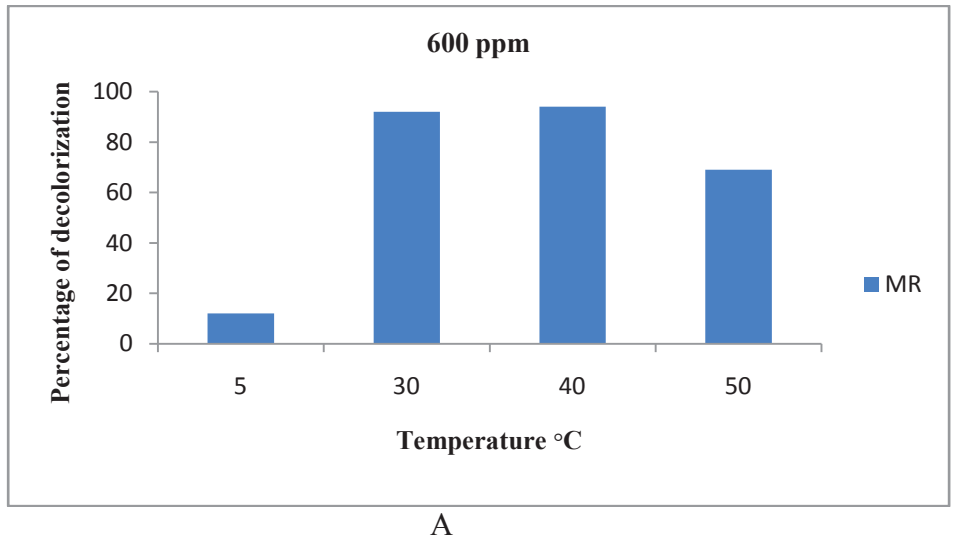
3.3. Decolorization test by using single organism

Decolorization of 800 mg/L of methyl red by three isolates (TA-1, TX-1 and TX-3) are shown in Fig. 1. All these isolates showed decolorization efficiency within 48 h incubation time. All isolates showed more or less similar trend in their decolorizing efficiency. However, isolate TX-1 showed a bit better decolorizing capacity than the other two isolates. Therefore, the isolate was selected for further studies. All of the three isolates grew well in liquid culture containing 800 mg/l methyl red in the presence of yeast extract and glucose and decolorize the azo-dyes. Decolorization of azo-dye, Direct Black 22, in presence of glucose and yeast extract has already been reported earlier (Mohana *et al*., 2008). Oforka and Oranusi (1978) stated the presence of easily metabolizable carbon source as an obligatory requirement for microbial decolorization of synthetic dye.

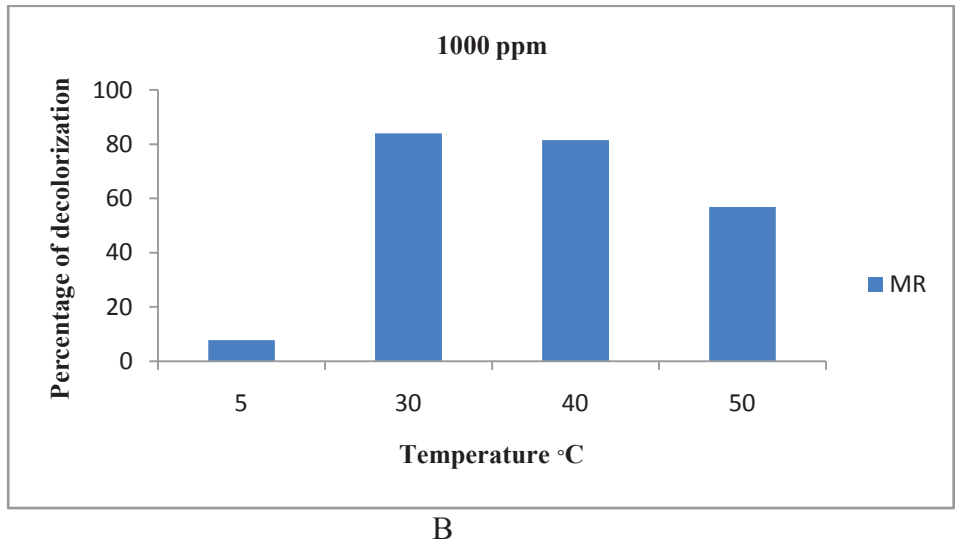
3.4. Decolorization of different concentrations of methyl red by Staphylococcus saprophyticus strain AUCASVE3

The results of the decolorization of various concentrations of methyl red by *S. saprophyticus* strain AUCASVE3 are presented in Fig. 2 A and B. The decolorization rates (%) of 600, 700, 800 and 1000 mg/L methyl red by the isolate *S. saprophyticus* strain AUCASVE3 were 94, 75, 79 and 67 (%), respectively for the first 24 h (Fig. 2 A). The decolorization rates of 600, 700, 800 and 1000 mg/L methyl red were 97, 82, 80, and 75(%) within 48 h, respectively (Fig. 2 B).

So *et al*. (1990) isolated *Acinetobacter liquefaciens* S-1 which decolorize and degrade methyl red into two colorless compounds namely 2-aminobenzoic acid (ABA) and N-N-dimethyl-ρ-phenylene diamine (DMPD), within 7 days and metabolized a maximum of 400 mg/L of methyl red in the presence of 5.0 g/L of glucose. Wong and Yuen (1996) isolated a bacterium Klebsiella pneumoniae RS-13, which has degraded methyl red 100 mg/L in presence of 0.5–5.0 g/L of glucose. Whereas in the present study, Staphylococcus saprophyticus strain AUCASVE3 showed 94 and 97 % methyl red (600 ppm) decolorization within 24 and 48 h, respectively.



A



B

Figure 3. Effect of temperature on decolorization of methyl red

3.5. Effect of temperature on methyl red decolorization

Effect of temperature on dye decolorization was studied and the results were shown in the Fig. 3 A and B. Better decolorization was observed between 30-40ºC temperatures by the isolate *Staphylococcus saprophyticus* strain AUCASVE3.

In another study, Moutaouakkil *et al*. (2003) observed that under optimal conditions, *Enterobacter agglomerans* decolorized 92% of 100 mg/L of methyl red within 6 h of incubation in synthetic medium at 37°C. The temperature ranging between 30-40ºC was fond to be suitable for the decolorization by *S. saprophyticus* strain AUCASVE3 and a further increase in the temperature drastically affected decolorization activity of bacterial culture.

Moreover, decolorization and degradation can also detoxify the effluent. Recent works have revealed the existence of a wide variety of microorganisms capable of decolorizing a wide range of dyes. So, the high methyl red decolorization rate and decolorization efficiency of *Staphylococcus saprophyticus* strain AUCASVE3 enable this bacterium to be used in the biological treatment of industrial effluent containing azo dyes.

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