MULTI-K NOWLEDGE ELECTRONIC COMPREHENSIVE JOURNAL FOR EDUCATION AND SCIENCE PUBLICATIONS (MECSJ)

ISSUE (17), Mar (2019)

ISSN: **2616-9185**

www.mecsj.com

Isolation and Characterization of Marine Bacteria Able to Degrade AZO Direct Red Dye 31 (DR 31)

Hala Ezzat Abou El-Hassayeb

Department of Marine Microbiology, National Institute of Oceanography and Fisheries, Alexandria, Egypt

dr.lola2010@yahoo.com

Abstract

This study presents the isolation of marine bacteria, from Abo Ramad, Sharm El Madfaa and shalateen, Red Sea , Egypt , capable to degrading an azo dye Direct Red 31 (DR 31). The 16S rDNA sequence and phenotypic characteristics indicated an isolated organism as *Pseudoalteromonas* sp p34 *(EU864258).* This strain exhibited complete Direct Red 31 (DR 31) (100mg/L) decolorization after 16 hours, where the maximum decolorizion was at 800 mg/L of dye after 40 hours with 62% decolorization under static condition. For color removal, the optimum temperature was from 25 to 40°C, where the optimum pH ranged from 6.0 to 9.0. Moreover, the present work indicates the cabability of strain *Pseudoalteromonas sp p34* to decolorize another four different azo dyes. Biodegradation of the azo dyes was confirmed by using UV-Vis spectral analysis

Keywords: Marine Bacteria, Azo dye, Direct Red 31, Decolorization.

Introduction

Azo dyes are one of a variety of artificial organic compounds that represent the majority of textile dyes produced and widely used in industries including paper, food, cosmetics, leather, pharmaceutical and dyeing industries [1]. The first commercially successful synthetic dye was discovered for practical uses in 1856.There are huge

ISSN: **2616-9185**

numbers of different dyes with an annual output of 7×10^5 metric tons worldwide available commercially[2-3]. The loss of dye depends on the class of the dye application, ranging from 2% while the loss of basic dyes to fifty percent in some activated sulfonic dyes, leading to severe pollution in aquatic environments near the dyeing industries [4-5]. The improper disposal of liquid textile dyes in aquatic ecosystems reduces the penetration of sunlight, which in turn reduces the concentration of dissolved oxygen, water quality, and photosynthetic activity resulting in toxic effects on marine plants and animals, causing serious environmental problems worldwide [6]. In addition, many azo dyes have a severe adverse effect in terms of biological and chemical oxygen and organic carbon [7].It can also cause serious illness in human health such as a damage of liver, the reproductive system, kidney, brain, and nervous system, nausea, and hemorrhage and mucous membranes [8]. A lot of artificial azo dyes besides their metabolites are carcinogenic, toxic, and mutagenic [9]. Therefore, it is essential to treat industrial sewages having azo dyes and their metabolites before their final liberation into the environment.

Due to the low efficiency, high cost, limited versatility, the handling of the waste generated and the interaction of other wastewater components, the major drawbacks of these procedures have been largely studied [10]. On the contrary, biological processes are more operative than technologies as they are more cost-effective, environmental friendly and do not produce large amounts of sludge. Reduction, oxidation, photolysis, chemical precipitation, flocculation, adsorption and electrochemical treatment are physicochemical methods that are used for the elimination of azo dyes [11]. Many microorganisms belongingsuch as bacteria, algae, actinomycetes and fungi have been reported for their ability to decolorize azo dyes[12].Many studies reported that bacteria have the ability of dye decolorization whichever individually or in consortia [13–16]. In contrast, fungi have been used to advance the biological processes for azo dyes, but the decolorization with moderate rate limits the activity of their decolorization process [17].

The present work aimed to isolate a bacterial strain with a significant capability to degrade Direct Red 31(DR 31) as a carbon source. In addition, various physiochemical experiments have been done to increase the efficiency of dye removal. Degradation of the dye was confirmed through UV-Vis spectral analysis before and after decolorization.

Materials and Methods

Culture Medium and dyes

The mineral salts medium (MSM) was prepared [18]. The MSM consisted of the following constituents (g/L): $2KH_2PO_4$ (2.0), $Na_2HPO_4 \cdot 2H_2O$ (1.0), NH_4NO_3 (0.50), Ca (NO_3) $2 \cdot 4H_2O$ (0.050), $MgC_{l2} \cdot 6H_2O$ (0.10), and $FeC_{l2} \cdot 4H_2O$ (0.0075) with 10mL of the element solution per liter. For the element solution (mg/L), a mixture of: $NiC_{l2} \cdot 6H_2O$ (2.0), $ZnSO_4 \cdot 7H_2O$ (10.0), $MnC_{l2} \cdot 4H_2O$ (3.0), $CoC_{l2} \cdot 6H_2O$ (1.0), $CuC_{l2} \cdot 2H_2O$ (1.0), H_3BO_3 (30.0), and $Na_2MoO_4 \cdot 2H_2O$ (3.0), were prepared. Further, MSM was blended with Direct Red 31(DR 31) with different concentrations. The pH was adjusted to 7.0 ± 0.2. The MSM with agar (1.9% w/v) was used for isolation of pure culture. The media were sterilized at 121∘C for 20min before use. five textile dyes; Direct Red 31(DR31), Direct Blue 90, Direct Brown 115 , Direct Green 26 and Direct Orange 39, were purchased from the textile industry. They are widely used in textile industries. Direct Red 31(DR 31) was used as a model azo dye in this study (Fig. 1).

Isolation of Dye Decolorizing Bacteria

Water samples were collected from 5 coastal sites of Abo Ramad, 11 coastal sites of Sharm El Madfaa and 12 coastal sites of shalateen, Red Sea, Egypt, and were brought to the laboratory. 10mL of water sample was added to 100mL of MSM broth containing Direct Red 31(DR 31) (100mg/L) and incubated at 30^0C for 2 weeks under static as well as shaking conditions. Then 10mL the decolorized culture flask was added to 100mL of MSM broth containing (DR 31) and incubated for 7 days under

static condition. 0.5mL of decolorized culture spread over the MSM agar plates containing (DR 31) and incubated at 30°C for 2 days. The obtained colonies formed were purified by streaking on agar medium. Each purified culture was tested for the ability of degrading dye. One hundred and thirty five isolates were obtained. The potential isolate was selected and characterized by using the biochemical and morphological properties [19].

Identification of Dye Decolorizing Bacteria based on16S rDNA Sequencing

Genomic DNAs of the selected isolate was extracted with the genomic DNA extraction protocol of Gene Jet genomic DNA purification Kit. The PCR thermocycler was set as follow: 95°C for 5 min for initial denaturation, 30 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 2 min and a finally at 72°C for 10 min. The product from PCR was made by Sigma Scientific Services Company, Egypt. Various alignments of the resulted sequences of the closely members were done by using BioEdit (software version 7). Sequences of rRNA genes were done by (NCBI) database [20].

Decolorization Experiment

100mL of sterilized MSM broth supplemented with Direct Red 31 (DR31) (100mg/L) and yeast extract (0.1% w/v). Yeast extract was added to increase the dye decolorization efficiency. They were inoculated with 5mL of cultures broth. The flasks were incubated at 30°C under static as well as shaking of 120 rpm, conditions. The supernatant was collected by centrifuging at 10,000 rpm for 15min. Decolorization and bacterial growth were estimated spectrophotometrically then the percentage was calculated [21]. Decolorization of dye was monitored using UV-Vis spectrophotometer.

Decolorization (%)

ISSN: **2616-9185**

$$= \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}}$$

Optimization of Physicochemical Parameters

The decolorization efficiency of *Pseudoalteromonas sp p34* on Direct Red 31(DR 31) was studied at different temperatures values (20,25,30,35,40,45,50∘C) and pH (4,5,6,7,8,9,10). The obtained optimum temperature at 30∘C and pH7.0 were for the decolorization activity under physicochemical factors such as salt concentration (1–6%), yeast extract concentration (0.1–2.0 g/L) and dye concentration (100–800mg/L). The decolorization of azo dye was carried out by incubating MSM containing Direct Red 31 (DR31) dye with bacterial strain *Pseudoalteromonas sp p34*.

Results and Discussion

Isolation, Screening of bacterial strains

One hundred and thirty five isolates were isolated. The potential bacterial strain *Pseudoalteromonas sp p34* showed a positive decolorization of Direct Red 31(DR 31) within 16 h under static condition. Its colony was white to light yellow, round, and slick. The potential strain was identified as *Pseudoalteromonas sp p34* on the basis of the biochemical characteristics and16S rDNA gene sequence. It was close to the member of *Pseudoalteromonas sp p34.group* [22] submitted to Genbank with accession number of *EU864258*. Some reports studied the degradation of polycyclic aromatic hydrocarbons by *Pseudoalteromonas sp p34* [23-24].

Effect of Shaking and Static Conditions

The decolorization efficiency of *Pseudoalteromonas sp p34* on Direct Red 31(DR 31) showed 100% decolorization under static condition when compared to only 22% decolorization was observed under shaking condition within 16 h. On the contrary, the

bacterial growth was lower under static condition as compared to shaking condition (Figure 2). Under shaking condition, electrons resulting by oxidation of electron donors from is used preferentially reduce free oxygen instead of azo dyes [25]. Bacteria cannot attack azo dyes, under aerobic conditions [26]. Similar studies were confirmed that by other researchers [14]. As a result, the following experiments were maintained under static conditions.

Effect of pH

Decolorization of Direct Red 31(DR 31) by *Pseudoalteromonas sp p34* was done by a range of pH 4.0 to 10.0. The potential isolate showed a high decolorization of 100% at pH 7.0. The percentage of decolorization decreased gradually from 96%, 63% and 41% at pH 8, pH 9 and pH10, respectively. While a severe decrease of percent decolorization from 88%, 49% and 14% at pH 6, pH5 and pH4, respectively, was found (Figure 3). Similar studies reported that the pH7 might be more suitable for azo dyes decolorization and for industrial applications [27].

Effect of Temperature

During the optimization of temperature, the maximum dye decolorization activity of *Pseudoalteromonas sp p34* was found by 100% at 30◦C after 16 h(Figure 4). The dye decolorization activity of *Pseudoalteromonas sp p34* was found with increase in temperature by 89% and 91% at 25 and 35◦C, respectively. By increasing the temperature, decolorization activity was decreased by 76% and 52% at 40◦C and 45◦C, respectively, decolorization activity of only 30% was showed at 50◦C. Similar results were indicated the same decrease in decolorization around 30◦C [16].This result indicates the effect of high temperature on enzyme activities [28].

Effect of Initial Direct Red 31 Dye Concentration

The percentage of decolorization of Direct Red 31(DR 31) by the *Pseudoalteromonas sp p34* was studied by a range of initial dye concentration from 100 to 800mg/L, gradually, the percentage of decolorization started to decrease with increasing the

ISSN: **2616-9185**

concentration of the dye (Figure 5). It could effectively decolorize up to 100mg/L of Direct Red 31 (DR31) (100%) within 16 h and is decreased to 62%, when dye concentration increased to 800 mg/L and decolorization time increases from 16 h to 40 h, respectively. At high concentration of dye, the percentage of decolorization of dye decrease due to the presence of sulfonic acid groups on Direct Red 31(DR31) which inhibit the growth of *Pseudoalteromonas sp p34* [14].

Effect of Salt Concentration

The percentage of decolorization of Direct Red 31(DR 31) by *Pseudoalteromonas sp p34* was studied by the effect of salt concentration by a range from 1 to 6% (w/v), gradually. It was observed that, the maximum decolorization was at 1 % salt concentration by 100% and started to decrease gradually by increasing the percentage of salt concentration after 16 h (Figure 6). This decreasing was observed as 96%, 72% and 42% of decolorization at 2%, 3% and 4% of salt concentration, respectively. Only 10% of decolorization dye was observed at 6% of salt concentration. Similar reports have been confirmed the same results about the effect of higher salt concentration on the percentage of decolorization of dye [29]. This is might be due to the higher salt concentration that cause inhibition of the bacterial activities. This bacterial inhibition was due to the loss of the activity of cells or plasmolysis at high salt concentration [30].

Effect of Different Yeast Extract Concentrations

Yeast extract considered as a better nitrogen source for decolorization of Direct Red 31(DR31) [14].The effect of yeast extract of different concentrations (0.1–2.0 g/L) on the decolorization of Direct Red 31(DR31) (100mg/L) by *Pseudoalteromonas sp p34*, was studied. It was observed that, decolorization dye started to increase by 55, 71 and 90%, respectively, by increasing concentration of yeast extract by 0.1, 0.25 and 0.5g/L. Addition of 1 g/L of yeast extract complete the decolorization of Direct Red 31(DR 31) by 100%. By increasing yeast extract concentration, there is no change on

the decolorization activity (Figure 7). Various nitrogen sources play an important role as they act as an electron donor in the decolorization of azo dyes.

Decolorization of Different Azo Dyes

Four different azo dyes were used in this study. *Pseudoalteromonas sp p34* was tested for its ability to decolorize four other different azo dyes such as Direct Blue 90, Direct Brown 115, Direct Green 26 and Direct Orange 39. The strain showed the ability to degrade all different azo dyes within 36 h (Table 2). The efficiency was 98, 94, 89 and 79% for Direct Blue 90, Direct Brown 115, Direct Green 26 and Direct Orange 39, respectively. The presence of sulfonic groups in the four tested dyes affect the variation of decolorization[31].

The decrease in peak at the respective absorption maximum of the studied azo dye suggests the cleavage of -N=N- bond by the anaerobic reductive degradation of the azo dye. However, we can observe a difference in UV-Vis spectral pattern before and after decolorization (Figure 8).

In conclusion, it can be concluded from the above study that, a bacterial strain *Pseudoalteromonas sp p34* is capable of degrading Direct Red 31(DR 31) as a carbon source of under static condition. The potential bacterial strain has the capability to decolorize Direct Red 31(DR 31) at the most suitable temperature is 25–40°C and pH 6.0–9.0, salt, and initial dye concentrations. Furthermore, strain *Pseudoalteromonas sp p34* had the ability to decolorize four other different azo dyes therefore, *Pseudoalteromonas sp p34* can be used for the decolorization of various azo dyes.

Acknowledgment

The author is thankful to The Institute of Graduate Studies & Research, University of Alexandria, Egypt, for encouragement, facilities and support.

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Tables

Table 1. Biochemical characterization of *Pseudoalteromonas* sp. P34. based on Biolog's third generation

Bacterial strain	Characteristics
Catalase	−
Urease	+
Citrate utilization	−
Glucose	+
Sorbitol	+
Adonitol	+
Esculin hydrolysis	−
Xylose	−
Sodium gluconate	+
Mannose	+
Inulin	+
Maltose	+
Oxidase	+

Fructose	+
Galactose	+
Glycerol	+
Trehalose	+
Sucrose	+
Salicin	+
Inositol	+
Mannitol	+
Arabitol	+
Erythritol	+
Rhamnose	+
Cellobiose	+
Melezitose	+
Xylitol	−
D-Arabinose	+
Sorbose	−

+: positive, and −: negative

Table 2 : Decolorization of different azo dyes by *Pseudoalteromonas sp p34.*

Dyes	**λmax (nm)**	**% Decolorization**	**Time (h)**

Direct Blue 90	*417*	*98*	*14*
Direct Brown 115	*536*	*94*	*20*
Direct Green 26	*540*	*89*	*26*
Direct Orange 39	*428*	*79*	*36*