

Exploring the potential of halophilic archaea for the decolorization of azo dyes

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ABSTRACT

Azo dyes are being extensively used in textile industries, so finding a proper solution to decolorize them is of high importance. In order to find azo dye decolorizing strains among haloarchaea, which are well known for their tolerance to harsh environmental conditions, fifteen haloarchaeal strains were screened. *Halogeometricum* sp. strain A and *Haloferax* sp. strain B with the highest decolorization ability (95% and 91% for Remazol black B; both about 60% for Acid blue 161, respectively) were selected for further studies. It was shown that both strains were able to grow and decolorize the dye in a medium containing up to 5 M NaCl, with optimum decolorization activity at 2.5–3.4 M, pH 7, and a wide temperature range between 30 to 45 °C. Moreover, both strains were able to tolerate and decolorize up to 1,000 mg l⁻¹ Remazol black B. Also, they were able to survive in 5,000 mg l⁻¹ of the dye after 20 days' incubation. Glucose and yeast extract were found to be the best carbon and nitrogen sources in the decolorization medium for both strains. This is the first report studying decolorization of azo dyes using halophilic archaea.

Key words | azo dye, biodecolorization, halophilic archaeon, Remazol black B

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INTRODUCTION

Nowadays, azo dyes, the largest group of the synthetic dyes with a great variety of colors, are extensively used in different industries including paper printing, food, pharmaceutical, cosmetic, and textile. The latter is the largest consumer of azo dyes; it is worth noting that during dyeing processes different amounts of the used dye depending on the structure, from 2% (w v⁻¹) for basic dyes to 50% (w v⁻¹) for reactive dyes, are released to the industrial effluents and finally to the environment (Pandey *et al.* 2007; Saratale *et al.* 2011b). The presence of dyes has a negative effect on terrestrial and aquatic ecosystems, because of their carcinogenicity and genotoxicity to organisms. They also disturb photosynthesis by impeding the penetration of the light into deeper layers and lowering the oxygen concentration (Saratale *et al.* 2011a).

Various attempts have been made to characterize textile wastewaters. A recent study by Kehinde & Aziz (2014) showed that pH and temperature could vary in different effluents from 6.95 to 11.8 and 21 to 62 °C, respectively. Furthermore, study on effluent samples of a textile industry in Brazil indicates that pH and temperature varies from

9.9–11.6 and 41–47 °C respectively. Moreover, 15–20% concentration of salt has been reported in dye-stuff industries wastewaters (Khalid *et al.* 2008; Cerqueira *et al.* 2009).

Azo dyes are usually recalcitrant to conventional wastewater treatment methods (Talarposhti *et al.* 2001); for example, physicochemical methods to eliminate azo dyes from effluents have serious limitations such as high cost, large amounts of sludge production, and inability to completely remove azo dyes from polluted effluents. Therefore, biodecolorization could be considered as a promising alternative to physicochemical methods, because of its environmental friendly nature and cost-effective properties (Talarposhti *et al.* 2001). Generally, two steps are considered for complete azo dye degradation by bacteria. The first step is the reductive cleavage of the azo bonds under anaerobic condition producing aromatic amines; this step should be followed by aerobic breakdown of the previously formed intermediates, which might be mutagen, carcinogen, and also more hazardous than primary azo dyes (Eslami *et al.* 2016).

It has been shown that many organisms including fungi, bacteria, yeast, algae, and plants are able to decolorize azo

dyes (Chen *et al.* 2003). Studies indicate that fungi need particular conditions for their ideal decolorization activity, such as low pH, which may limit their application. Recently, studies have proven that halotolerant and halophilic bacteria have potential biotechnological applications and could be useful in decolorization of dyes in the presence of nitrate, sulfate, chloride, and carbonate salts, which are commonly used during reactive dyeing processes (Asad *et al.* 2007; Amoozegar *et al.* 2011; Pourbabae *et al.* 2011). Haloarchaea have been used in several biotechnological applications such as degradation of toxic materials (Oren 2010), liposome construction (Galinski *et al.* 1992), and food fermentation (Oren 2010); however, there is no report considering their potential for azo dye decolorization. Whereas textile industries are commonly involved with harsh conditions like high temperature and salt concentrations (sometimes reaching to 150–200 g l⁻¹ in wastewaters) (Tan *et al.* 2009), halophilic archaea might be suitable candidates for decolorization in this aspect. Therefore, discovering new strains of halophilic archaea which are capable of decolorizing azo dyes in the harsh environment of textile wastewater could be of great importance and a novel procedure in the matter.

In the present work, decolorization ability of 15 isolated haloarchaea toward different azo dyes was measured. Two strains with the highest decolorization results were selected for further studies. The effect of different environmental factors on the growth and decolorization were also determined. Moreover, azo dye decolorization was investigated using whole archaeal cells instead of isolated enzymes, because of some advantages. The use of whole cells in industrial units is more affordable than using purified enzymes, and intact cells protect intracellular enzymes from the harsh conditions of wastewaters (Pearce *et al.* 2003). Because of the promising decolorization results achieved by studying selected haloarchaea strains, they may be considered as good candidates for treating the textile and dyeing industries wastewaters. To the best of our knowledge, this is the first report exploring azo dye decolorization potential of halophilic archaea.

METHODS

Chemicals

Azo dyes including Remazol black B, Reactive red ME 43, Reactive yellow MER 145, Reactive blue SSB 222, Acid blue 161, Methyl red, Pigment yellow KBF, and Scarlet

sulphonyl BNLE were provided from Ciba Geigy GmbH representative in Iran (CIBA). Culture media components and solvents were Merck products. Rest of the chemicals were of analytical grade purity.

Archaeal strains and culture conditions

Fifteen different strains isolated from a saline cave located in Qeshm island in Persian gulf were screened to find azo dye decolorizing ones. In order to isolate halophilic archaeal strains from the saline cave, samples were collected from water and salt sediments. Samples were diluted and cultured on modified growth medium 23% (MGM), a saline medium that is specific for archaeal growth and isolation, and incubated at 40 °C for about one month. Then the streak plate method on MGM agar was used to isolate pure strains. The MGM (pH 7.2 ± 0.1) with 23% (v w⁻¹) total salts contained (in g l⁻¹) 183.9 NaCl, 22.9 MgCl₂, 26.8 MgSO₄, 5.38 KCl, 1 peptone, and 0.2 yeast extract (Dyall-Smith 2009).

Molecular identification of isolated strains

After finding the strains that performed decolorization, these strains were identified through 16 rRNA gene sequencing. To this purpose, the genomic DNA of the strains was extracted (Marmur 1961) and 16S rRNA genes were amplified using 20F 5' TCCGGTTGATCCTGCCG 3' and 1530R 5' AGGATCAAGTGTCGGGGTGCG 3' universal primers, according to the following program: initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min, and final extension at 72 °C for 7 min. The purified PCR products were sequenced by Bio-neer company (Korea) and the sequence similarity searches were done using EzTaxon-e server (Kim *et al.* 2012), and the phylogenetic tree was constructed according to the neighbor-joining algorithm using MEGA software version 6 for selected strains (Tamura *et al.* 2013).

Culture conditions for decolorization

Decolorization experiments were carried out in culture tubes containing MGM broth with 3 M NaCl (pH 7.2) supplemented with 50 mg l⁻¹ Remazol black B (di-azo dye) or Acid blue 161 (mono-azo dye). 5% (v v⁻¹) of inoculant (equivalent to 0.5 McFarland standard) from all strains were cultured in MGM separately and incubated at 40 °C in static condition (no shaking). Growth and decolorization ability were evaluated up to seven days. For each

measurement, samples were taken from the cultured medium and growth was determined based on the difference between the OD₆₂₀ of culture samples, before and after centrifugation, using Shimadzu UV-106A spectrophotometer (the path length of the cuvette was 1 cm) (Dong *et al.* 2003). To calculate decolorization percent, the absorbance of the clarified decolorization media was determined at λ_{max} of the dye (600 nm for Remazol black B and 610 for Acid blue 161) (Asad *et al.* 2007) and decolorization efficiency was expressed as:

$$\% \text{ Decolorization} = \frac{(\text{Initial absorbance} - \text{Sample absorbance})}{\text{Initial absorbance}} \times 100$$

Abiotic control reactions were used to assure that decolorization was only due to microbial activity and not because of the variation in parameters or light oxidation.

Effect of different culture conditions on strains growth and decolorization of Remazol black B

In all experiments given below, 5% (v v⁻¹) of inoculant (equivalent to 0.5 McFarland standard) was added to MGM and cultures were incubated at 40 °C. The effect of different factors including aeration, temperature (30, 35, 40, 45 and 50 °C), pH (5–9), and concentration of Remazol black B (50 to 5,000 mg l⁻¹) on growth and decolorization by selected strains was studied in liquid MGM. To examine aeration effect on decolorization of Remazol black B, cultures were subjected to shaking (150 rpm) and static condition.

Effect of salts (NaCl, KCl, NaNO₃, and KNO₃), different nitrogen (peptone from soy and meat, yeast extract, ammonium sulfate, and urea), and carbon sources (glucose, sucrose, maltose, lactose, propionic acid, acetate sodium, and citric acid) was also studied in MGM, each time by replacing one related component in the medium with above mentioned salts, carbon, or nitrogen sources. To evaluate effect of different salts, NaCl in MGM was replaced by 3 M of KCl, NaNO₃, or KNO₃. Effect of different concentrations of NaCl was studied by adding 2.1–5.1 M of NaCl to the medium.

MGM supplemented with 5,000 mg l⁻¹ dye was also used to study the dye toxicity tolerance of strains. To this purpose, the cultures were centrifuged and precipitated cells were cultured on MGM agar without dye after 20 days of incubation.

Dye consumption as the sole carbon source

To evaluate the strains' ability to use Remazol black B as the sole carbon source, MGM was modified and peptone and yeast extract (carbon sources) were omitted from MGM. The modified MGM contained basal salts of MGM, 50 mg l⁻¹ Remazol black B, and one of these nitrogen sources: 0.05% (w v⁻¹) yeast extract, 1% (w v⁻¹) ammonium chloride, or 1% (w v⁻¹) sodium nitrate. The strains were cultured onto the modified MGM and the growth was considered as the dye consumption for carbon source.

Decolorization of other dyes

Decolorization of mixed dyes in MGM with 10 mg l⁻¹ of each dye including Remazol black B, Direct blue 161, Reactive red, Reactive blue, and Reactive yellow by selected strains was evaluated. To this purpose, strains were cultured in MGM broth with 3 M NaCl (pH 7) supplemented with mixed dyes, and then incubated at 40 °C for 7 days. Decolorization was determined by measuring the OD₆₀₀ of sample supernatants.

To observe decolorization ability of selected strains for different dyes, Reactive red, Reactive blue, Reactive yellow, Pigment yellow, Methyl Red, and Scarlet sulphonyl BNLE were added to the MGM separately, according to the above mentioned conditions for mixed dyes. Decolorization was determined by measuring the OD of samples supernatant at maximum wavelength of each dye.

RESULTS AND DISCUSSION

Dye decolorization screening

As mentioned in the methods section, total of 15 different haloarchaea strains (namely A to O) were screened for decolorization (Tables 1 and 2). According to the results, strains A and B were the best decolorizing strains. As shown in Table 1, strains A and B decolorized Remazol black B to more than 70 and 90% after 4 and 7 days of incubation, respectively. Additionally, Acid blue 161 was decolorized to approximately 20% only by strain A after 4 days of incubation. However, both strains decolorized Acid blue 161 about 60% in the 7th day of incubation (Table 2).

There are few reports from halophilic and halotolerant bacteria such as *Halomonas* spp. (Asad *et al.* 2007; Guo *et al.* 2008) and *Halobacillus* sp. C-22 (Demirci *et al.* 2011) describing azo dye decolorization. As data from azo dye

Table 1 | Decolorization (%) of Remazol black B by isolated halophilic archaea. Values are averages of three independent experiment \pm standard deviations

Strain	Most similar type strain	Similarity ^a	3rd day	4th day	5th day	6th day	7th day
A	<i>Hgm. borinquense</i> DSM11551 ^(T)	99.1	66 \pm 3.4	73 \pm 1	73 \pm 3.6	84 \pm 4.1	95 \pm 2.3
B	<i>Hfx. mediterranei</i> CGMCC1.2087 ^(T)	99.4	72 \pm 2.1	79 \pm 1	84 \pm 2.3	84 \pm 1	91 \pm 2
C	<i>Haloarcula salaria</i> HST01-2R ^(T)	97.4	34 \pm 2.6	54 \pm 1	63 \pm 2	64 \pm 1	64 \pm 2.1
D	<i>Har. salaria</i> HST01-2R ^(T)	97.1	23 \pm 8.5	39 \pm 6.4	61 \pm 8.9	63 \pm 6.2	64 \pm 6.4
E	<i>Hfx. alexandrius</i> TM ^(T)	99.8	65 \pm 2	71 \pm 1	86 \pm 2.1	86 \pm 2.3	86 \pm 2
F	<i>Har. marismortui</i> ATCC43049 ^(T)	97.3	58 \pm 1	59 \pm 1	70 \pm 1	82 \pm 1	84 \pm 1
G	<i>Har. japonica</i> JCM 7785 ^(T)	98.7	18 \pm 3.5	18 \pm 3.6	43 \pm 4	56 \pm 3.4	57 \pm 4
H	<i>Hgm. borinquense</i> DSM11551 ^(T)	99	70 \pm 2	70 \pm 1	71 \pm 1	71 \pm 1	86 \pm 1
I	<i>Hfx. mediterranei</i> CGMCC1.2087 ^(T)	99.3	65 \pm 5.4	70 \pm 6.2	70 \pm 6.5	71 \pm 4.1	71 \pm 6.4
J	<i>Har. salaria</i> HST01-2R ^(T)	98	34 \pm 1	51 \pm 2	68 \pm 2	69 \pm 2.6	69 \pm 2.1
K	<i>Har. hispanica</i> ATCC33960 ^(T)	97.8	50 \pm 5.4	67 \pm 6.4	67 \pm 8.5	67 \pm 6.4	67 \pm 8.5
L	<i>Har. hispanica</i> ATCC33960 ^(T)	99.6	62 \pm 2	63 \pm 2	70 \pm 2	77 \pm 1	89 \pm 1
M	<i>Hfx. prahovense</i> TL6 ^(T)	100	59 \pm 0	78 \pm 1	78 \pm 2.1	81 \pm 2.3	85 \pm 2
N	<i>Hfx. alexandrius</i> TM ^(T)	99.9	62 \pm 2	68 \pm 1	77 \pm 1	80 \pm 2	89 \pm 2
O	<i>Halococcus morrhuae</i> ATCC17082 ^(T)	98.5	0	0	0	0	0

^aSimilarity between isolated strains and type strains determined based on 16S rRNA gene sequence analysis of strains.

Table 2 | Decolorization (%) of Acid blue 161 by isolated strains. Values are averages of three independent experiments \pm standard deviations

Strain	Most similar type strain	3rd day	4th day	5th day	6th day	7th day
E	<i>Hfx. alexandrius</i> TM ^(T)	0	0	0	19 \pm 1	44.3 \pm 1
K	<i>Har. hispanica</i> ATCC33960 ^(T)	0	0	0	23.3 \pm 2	23.6 \pm 0.5
A	<i>Hgm. borinquense</i> DSM11551 ^(T)	18 \pm 2.3	19.6 \pm 2	37 \pm 2	53.3 \pm 1	70 \pm 3
B	<i>Hfx. mediterranei</i> CGMCC 1.2087 ^(T)	0	0	45.3 \pm 3.3	46.6 \pm 1	68 \pm 1

decolorizing haloarchaea have not been obtained until present study, results from studies on decolorization ability of haloarchaeal strains in this work have been compared to data reported in literatures related to decolorizing bacteria.

Identification of archaeal strains

Phylogenetic identification results of the archaea used in this survey are represented in Table 1. According to the decolorization screening step outcomes, eight archaeal strains were able to decolorize the azo dye. Decolorizing strains belonged to the following genera: *Halogeometricum*, *Haloferax*, *Haloarcula*, *Halococcus*, *Haloarchaeobius*, *Halovivax*, *Halopenitus*, and *Halorientalis* (Table 1). The phylogenetic relationship between two selected archaeal strains *Hgm. borinquense* sp. strain A and *Hfx. mediterranei* sp. strain B and other related archaea is shown in Figure 1.

Effect of various factors on decolorization of Remazol black B

Effect of different factors on decolorization of Remazol black B and growth of the strains A and B was studied. The maximum decolorization percentage was the criterion for deciding on the best condition for decolorization. In this part, the lowest growth amount was important because in the practical application, the cell mass should be collected before release to the environment, so the lowest growth with the highest decolorization is desirable.

Effect of temperature and pH on strains growth and decolorization of Remazol black B

Optimum decolorization results and growth of *Hgm. borinquense* sp. strain A were at pH 7; and acidic or alkaline pH

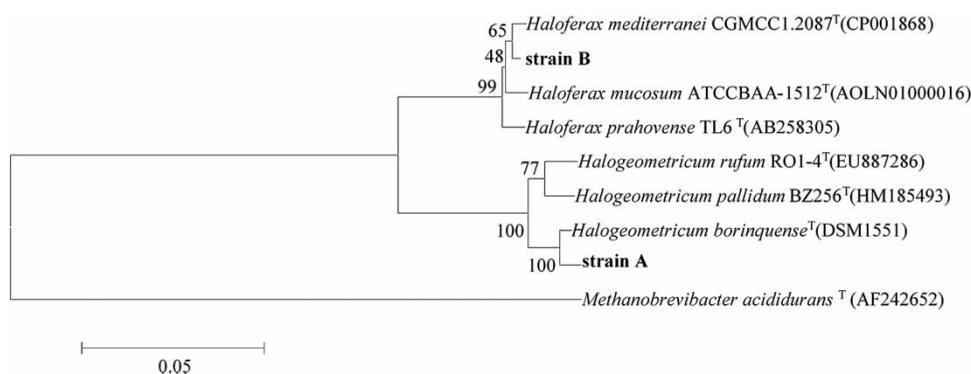


Figure 1 | Phylogenetic tree of *Haloferax* sp. strain B and *Halogeometricum* sp. strain A and related genera. The tree was constructed according to the neighbour-joining method. The scale bar represents 0.05 substitutions per nucleotide position and the numbers represent bootstrap values (%) indicating the accuracy of almost any statistical estimate in phylogenetic tree.

values showed negative effect on both decolorization ability and growth of the strain. Similar results were obtained for *Hfx. mediterranei* sp. strain B, but unlike strain A, it was able to decolorized Remazol black B at pH 10. Effect of pH on decolorization of Remazol black B is shown in Figure 2.

The effect of temperature on decolorization of Remazol black B and growth of the selected strains is shown in Figure 3. It could be inferred that both decolorization and

growth of the strains were increased with the temperature rise. The maximum decolorization for *Hgm. borinquense* sp. strain A was achieved at 45 °C while in the case of *Hfx. mediterranei* sp. strain B, temperature ranging from 40–50 °C was optimum. Both decolorization and growth of *Hgm. borinquense* sp. strain A were declined at 50 °C.

The observed results could be attributed to the increase in enzyme activity and archaeal growth with the temperature rise. Dafale et al. (2008) observed similar results for the decolorization of Remazol black B by a bacterial consortium. Decolorization increased with a temperature increase from 20 to 37 °C, while higher temperatures decreased the

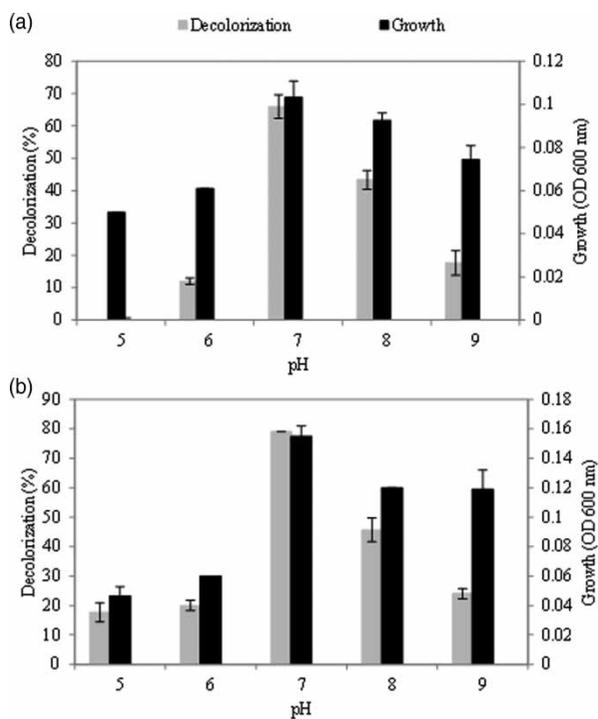


Figure 2 | Effect of pH on growth and decolorization activity of two selected haloarchaeal strains. Measurements were done after 4-day incubation at 40 °C. Results represent the means of three separate experiments and bars indicate \pm standard deviation.

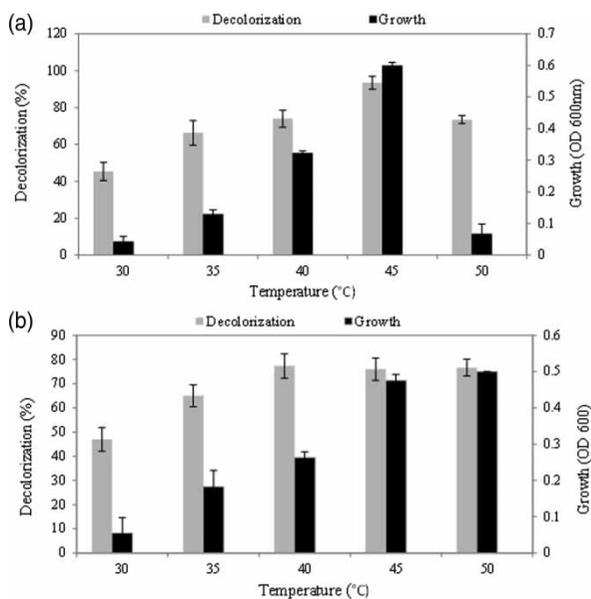


Figure 3 | Effect of temperature on decolorization activity and growth of two selected haloarchaeal strains. pH of MGM was adjusted to 7 and measurements were done after 4-day incubation. Results represent the means of three separate experiments, and bars indicate \pm standard deviation.

decolorization rate. Wang et al. (2009) studied decolorization of Remazol black B by *Enterobacter* sp. EC3. Again, decolorization increased as the temperature increased from 22 to 37 °C and decreased at higher temperatures. Our optimum decolorization temperature for both strains A and B was higher than the reported optimum temperatures for decolorization of Remazol black B by *Enterobacter* sp. EC3 (37 °C) (Wang et al. 2009) and *Bacillus* sp. strain YZU1 (40 °C) (Wang et al. 2013). Wang et al. (2013) suggested that the negative effect of an excessive increase in temperature on the decolorization rate by microorganisms is due to cell death or inactivation of enzymes involved in dye decolorization.

Effect of different salts and different NaCl concentrations on strains growth and decolorization of Remazol black B

For both *Hgm. borinquense* sp. strain A and *Hfx. mediterranei* sp. strain B, NaCl was the best salt to support growth and decolorization. By replacing NaCl with other salts, both growth and decolorization ability of strains were negatively affected (Figure 4).

Effect of different NaCl concentrations on growth and decolorization ability of selected strains is shown in Figure 5. Since both strains are halophilic archaea, NaCl is necessary for their growth and dye decolorizing activity; NaCl concentrations of 2.5–3.4 M resulted in the maximum growth and decolorization activity for both strains. Observations could

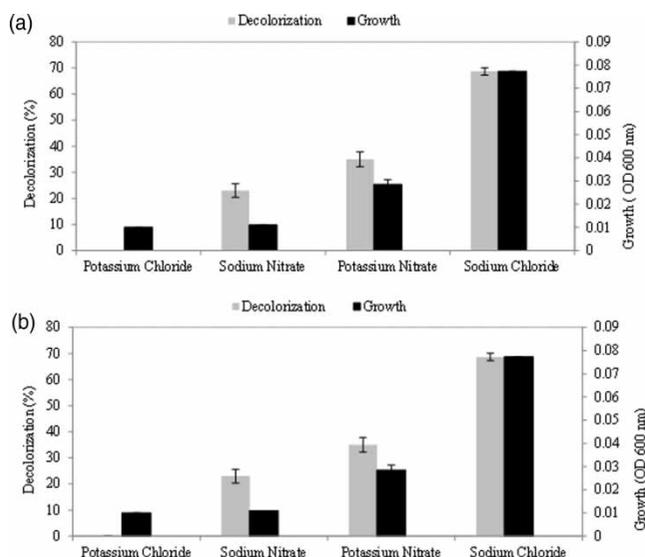


Figure 4 | Effect of salts on growth and decolorization activity of two selected haloarchaeal strains. pH of MGM was adjusted to 7 and measurements were done after 4-day incubation at 40 °C. Results represent the means of three separate experiments, and bars indicate ± standard deviation.

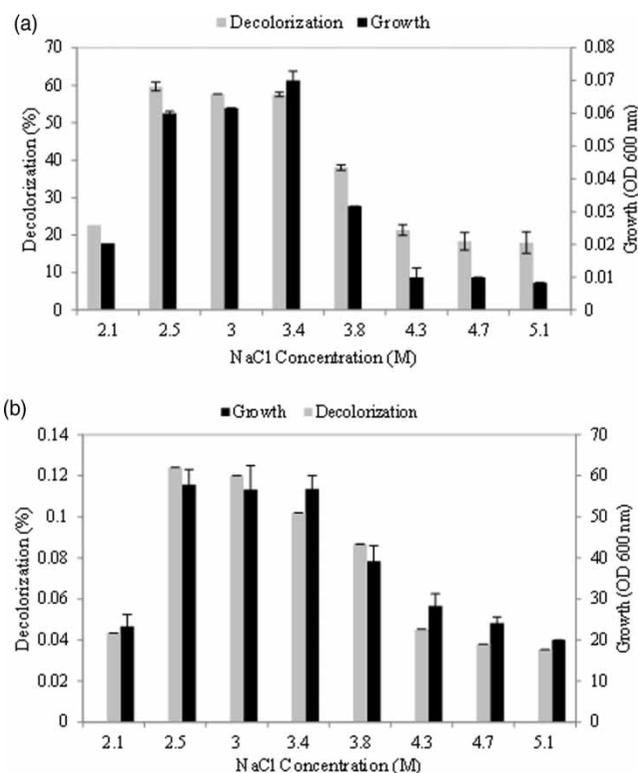


Figure 5 | Effect of NaCl concentrations on growth and decolorization activity of two selected haloarchaeal strains. pH of MGM was adjusted to 7 and measurements were done after 4-day incubation at 40 °C. Results represent the means of three separate experiments, and bars indicate ± standard deviation.

be attributed to the increased stability and activity of the haploidal enzymes, in addition to the improved cellular growth at the mentioned NaCl range. The effect of salinity on azo dye decolorization has been previously reported for bacteria; for example, *Shewanella putrefaciens* strain AS96 decolorized Reactive black 5 in a medium containing 0–60 g l⁻¹ NaCl (Khalid et al. 2008) and two other *Shewanella* species, *S. algae* and *S. marisflavi* were able to decolorize two other azo dyes in salinity of 50 g l⁻¹ (Khalid et al. 2008). For *Bacillus* sp. strain YZU1, the optimum decolorization of Reactive black 5 by the strain was in a medium containing less than 2 g l⁻¹ NaCl (Wang et al. 2013). Different strains of *Halomonas* showed decolorization of Remazol black B in salinities up to 200 g l⁻¹ NaCl (Asad et al. 2007) and *Klebsiella* sp. strain Y3 decolorized Methyl Red in the presence of 10–40 g l⁻¹ NaCl (Cui et al. 2014).

Effect of static or aerobic conditions on decolorization of Remazol black B by strains

Effect of aeration on decolorization showed that *Haloferax* sp. strain B decolorized Remazol black B in static condition

better than aerobic condition (95% compared to 75%). Likewise, *Halogeometricum* sp. strain A decolorized the dye in both static and aerobic conditions (95% compared to 85%) (data not shown). Given that oxygen is a better electron acceptor than azo group, decolorization of azo dyes is preferable in anaerobic conditions (Wang et al. 2009). Asad et al. (2007) reported that different *Halomonas* strains decolorized Remazol black B in static and anaerobic conditions while aeration inhibited the azo dye decolorization. Decolorization of Remazol black B by *Enterobacter* sp. EC3 occurred in both aerobic and anaerobic conditions, with better results in anaerobic one (Wang et al. 2009). It seems that *Halogeometricum* sp. strain A with almost equal decolorization ability at different aeration conditions could be more advantageous for wastewater treatment processes.

Effect of nutritional factors and different concentrations of Remazol black B on strains growth and decolorization of Remazol black B

Effect of different carbon and nitrogen sources on growth and decolorization of Remazol black B by selected strains

is shown in Tables 3 and 4. According to the results, sucrose and glucose were the best carbon sources and yeast extract was the best nitrogen source for growth and dye decolorization by *Hgm. borinquense* sp. strain A. For *Hfx. mediterranei* sp. strain B, glucose as a carbon source and yeast extract as a nitrogen source showed the best results (Tables 3 and 4). Similar to these findings, Padamavathy (2003) reported that decolorization of Remazol black B by a bacterial consortia in the presence of glucose as the sole carbon source in the culture medium was more than other tested carbon sources. Likewise, *Enterobacter* sp. EC3 decolorized Remazol black B more efficiently when glucose was added to its culture medium (Wang et al. 2009). In contrast to the present study, glucose showed inhibitory effect on decolorization of azo dyes by *Shewanella* sp. (Khalid et al. 2008).

As previously mentioned, yeast extract was the best nitrogen source for growth and decolorization of Remazol black B by both strains A and B. Similar to these findings, decolorization of azo dyes by *Shewanella* sp. was maximum when yeast extract was used as a nitrogen source (Khalid et al. 2008).

Table 3 | Decolorization (%) of azo dye in various carbon sources. Values are averages of three independent experiments \pm standard deviations

Factors	Growth (OD620 nm)		% Decolorization (OD600 nm)	
	Strain A	Strain B	Strain A	Strain B
Carbon sources:				
Sucrose	0.08 \pm 0	0.25 \pm 0.01	76 \pm 5.6	62.6 \pm 1.4
Glucose	0.1 \pm 0	0.31 \pm 0.02	83.5 \pm 6.3	81.5 \pm 4.9
Maltose	0.1 \pm 0	0.38 \pm 0	65 \pm 0	77 \pm 0.7
Lactose	0.06 \pm 0.01	0.20 \pm 0	47.5 \pm 3.5	67.5 \pm 0.7
Propionic acid	0.07 \pm 0	0.18 \pm 0.01	56.6 \pm 0.5	70.6 \pm 0.18
Citric acid	0.06 \pm 0	0.05 \pm 0	0.01 \pm 0	20.66 \pm 0.7
Acetate sodium	0.1 \pm 0	0.26 \pm 0.02	67 \pm 0	72 \pm 0.26

Table 4 | Decolorization (%) of azo dye in various nitrogen sources. Values are averages of three independent experiments \pm standard deviations

Factors	Growth (OD620 nm)		% Decolorization (OD600 nm)	
	Strain A	Strain B	Strain A	Strain B
Nitrogen sources:				
Yeast extract	0.12 \pm 0	0.31 \pm 0.02	80 \pm 2.1	81 \pm 0
Peptone from soy	0.05 \pm 0	0.11 \pm 0	64.3 \pm 6.3	63.5 \pm 1.3
Peptone from meat	0.1 \pm 0	0.18 \pm 0	65.5 \pm 0.4	70 \pm 0
Ammonium sulfate	0.0	0.0	0.0	0.0
Urea	0.04 \pm 0	0.03 \pm 0	32.3 \pm 2.8	32.5 \pm 1.3

Table 5 | Decolorization (%) of azo dye in various dye concentrations. Values are averages of three independent experiments \pm standard deviations

Factors	Growth (OD ₆₂₀ nm)		% Decolorization (OD ₆₀₀ nm)	
	Strain A	Strain B	Strain A	Strain B
Concentration of dye (mg l ⁻¹)				
400	0.04 \pm 0	0.07 \pm 2.8	93.6 \pm 3.2	86.6 \pm 0
600	0.02 \pm 0	0.05 \pm 1.7	87.3 \pm 3	75 \pm 0
800	0.01 \pm 0	0.03 \pm 4	80.6 \pm 2	53.6 \pm 0
1,000	0.008 \pm 0	0.01 \pm 0	61.6 \pm 2.8	46.3 \pm 0
2,000	0	0	0	0
5,000	0	0	0	0

Increasing the concentration of Remazol black B showed negative effect on growth and decolorization ability of both *Hgm. borinquense* sp. strain A and *Hfx. mediterranei* sp. strain B. Optimum dye concentrations for growth and decolorization activity of *Hgm. borinquense* sp. strain A and *Hfx. mediterranei* sp. strain B were determined to be 600 and 400 mg l⁻¹, respectively.

Both strains decolorized the dye up to 1,000 mg l⁻¹. No decolorization was observed in 2,000 and 5,000 mg l⁻¹ of the dye (Table 5). Moreover, examining dye toxicity tolerance of selected strains revealed that both strains were able to survive after 20 days incubation in 5,000 mg l⁻¹ of dye.

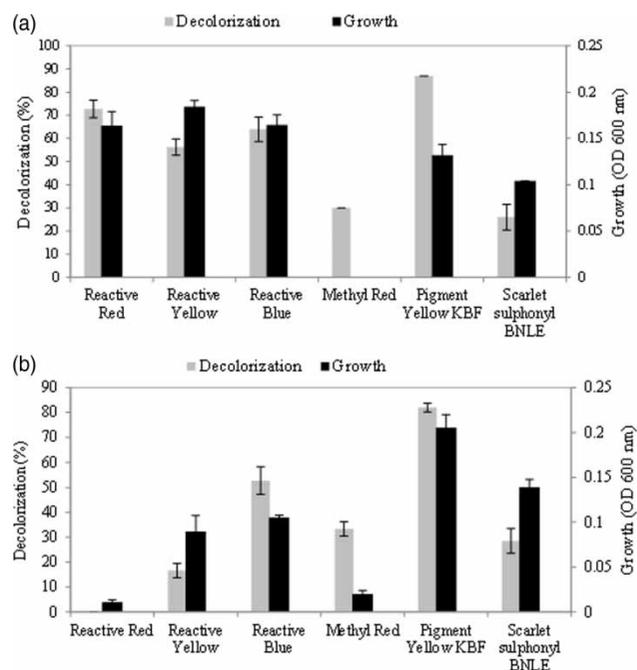
Remazol black B as the sole carbon source

The results showed that the selected strains could not decolorize Remazol black B as the sole carbon source in the case of using ammonium chloride and sodium nitrate as nitrogen sources, but 0.05% yeast extract was the lowest concentration of yeast extract at which both selected strains were able to decolorize the dye up to 50%. As nitrogen is not the only nutrition source in yeast extract, it seems likely that something other than nitrogen is promoting decolorization and therefore yeast extract is not necessarily the best source of nitrogen.

Decolorization of mixed dyes and different dyes

After one week incubation of selected strains in the presence of mixed dyes, *Hfx. mediterranei* sp. strain B was capable of decolorizing 75% of the mixed dyes; however, *Hgm. borinquense* sp. strain A was not able to decolorize this mixture of dyes.

Both strains were tested to decolorize individual six different azo dyes (Figure 6). *Hgm. borinquense* sp. strain A and *Hfx. mediterranei* sp. strain B showed decolorization ability towards Pigment yellow KBF (90 and 80%), Reactive blue SSB 222 (67 and 53%), Methyl red ME 43 (28 and 35%), Scarlet sulphonyl BNLE (28 and 30%), and Reactive yellow MER (58 and 18%), respectively. Although *Hgm. borinquense* sp. strain A could readily decolorize 72% of Reactive red ME 43, *Hfx. mediterranei* sp. strain B could not decrease the dye absorbance.

**Figure 6** | Decolorization (%) and growth of two selected strains at 50 mg l⁻¹ of different azo dyes. pH of MGM was adjusted to 7 and measurements were done after 4-day incubation at 40 °C.

CONCLUSIONS

The azo dye decolorization potential of 15 halophilic archaea was explored in this study. Valuable characteristics of both best decolorizing haloarchaea strains toward dye decolorization at different environmental conditions including pH, temperature, and salinity along with their ability to survive and decolorize increased amounts of the dye make them good candidates for dye-removal processes from textile effluents. In fact, the haloarchaea ability to decolorize dyes at significantly higher temperatures and salt concentrations than bacteria is another advantage for using haloarchaea in this field. Since there are different dyes used in textile industries releasing to the wastewaters, the potential to decolorize mixed dyes and also decolorize different dyes individually is another important characteristic for the treatment process. In sum, high decolorization activity against azo dyes along with the ability to decolorize mixed-dyes by halophilic archaea are valuable results which were observed for the first time in this study.

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