Biodegradation (2008) 19:15–19
DOI 10.1007/s10532-007-9110-1

ORIGINAL PAPER

A novel moderately halophilic bacterium for decolorizing azo dye under high salt condition

Jianbo Guo · Jiti Zhou · Dong Wang · Cunping Tian · Ping Wang · M. Salah Uddin

Received: 18 October 2006 / Accepted: 14 February 2007 / Published online: 9 March 2007

Abstract *Halomonas* sp strain GTW was newly isolated from coastal sediments contaminated by chemical wastewater and was identified to be a member of the genus *Halomonas* by 16S rDNA sequence analysis and physical and biochemical tests. The optimal decolorization conditions were as follows: temperature 30°C, pH 6.5.0–8.5, NaCl 10–20% (w/v) and the optimal carbon source was yeast exact. The results of experiments demonstrated that the bacteria could decolorize different azo dyes under high salt concentration conditions, and the decolorization rate of five tested azo dyes could be above 90% in 24 h. The exploitation of the salt-tolerant bacteria in the bio-treatment system would be a great improvement of conventional biological treatment systems and the bio-treatment concept.

Keywords Halophilic bacteria · Decolorization · *Halomonas* · Azo dyes

J. Guo (✉) · J. Zhou · D. Wang · C. Tian · P. Wang · M. S. Uddin
School of Environmental and Biological Science and Technology, Dalian University of Technology, Dalian 116023, P.R. China
e-mail: jianbguo@yahoo.com.cn

J. Guo
School of Environmental Science and Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, P.R. China

Introduction

Hyper-salinity chemical industry wastewaters often contain a range of chemicals, which are recalcitrant to biodegradation. For example, Reactive dyes are very soluble by design and, as a result, not all are used up by textile fibers during the dyeing process and therefore end up with the discharge from dye houses (Pearcea et al. 2003). At the same time, high salt concentration is also a consequence product of batch processes both in the dye manufacturing industries and in the dye-consuming industries, and the salt concentration is up to 15–20%. Textile wastewaters are complex waste products containing dyes, sizing agents and dyeing aids that characterized by their deep color and high salt concentration (EPA 1997; Carliell et al. 1994; Manu and Chaudhari 2003).

Generally, sodium concentration above 3 g l^{-1} can cause moderate inhibition of most bacterial activities (De Baere et al. 1984). Hyper-salinity wastewater usually causes plasmolysis and/or loss of activity of cells, therefore, some traditional aerobic- and anaerobic-biological treatments result in low BOD (biological oxygen demand) removal performance, or activated sludge is usually protected from high salinity by pre-treatment of the influent wastewater. At the same time, many microorganisms could thrive in saline environments. Therefore, exploitation of the salt-tolerant bacteria in the bio-treatment system

would be a great improvement of conventional biological treatment systems and the bio-treatment concept.

This paper describes a study on the isolation and identification of a salt-tolerant bacterium used to decolorize azo dyes under high salt concentration conditions.

Materials and methods

Dyes and reagents

The dyes used in this study were from Dye Synthesize Laboratory, Dalian University of Technology. All other reagents were analytical grade and were purchased from Shenlian Ltd. (Dalian, China).

Isolation and characterization of decolorization and salt-tolerant bacteria

Stain GTW was isolated from the coastal sediment in Dalian Bay, China and deposited in the China General Microorganism Culture Center (CGMCC) with the accession number 1527. It was a Gram-negative, non-motile, facultative, rod-shaped bacterium measuring 0.5–0.7 μm wide and (1.6–2.0) μm. long. No flagellum was found by electron microscopy. Colonies were smooth, glistening, circular, low-convex and yellow. It was oxidase-positive and catalase-positive. Acid could be produced from glucose, sucrose, lactose and fructose, and starch was hydrolyzed.

The salt-tolerant medium (STM) was used both for enrichment and pure cultures of decolorization and salt-tolerant organisms. The medium contained yeast extract 5 g l^{-1}, peptone 10 g l^{-1} and 0–250 g l^{-1} NaCl (pH 7.0). Anaerobic decolorization condition was conducted as described in our previous report (Guo et al. 2005).

Coastal sediment samples were collected from Dalian bay, China, which was contaminated with chemical wastewater.

Genomic DNA extraction, PCR

The genomic DNA was extracted from the cells (without any pre-treatment) by the method described previously (Mufiel et al. 1999). The universal primers 8f and 1522r were used and the PCR amplification of 16 S rDNA was conducted in a total volume of 50 μl, containing 1μl of each primer, 4μl (each) dNTP, 5μl 10 × LA-taq buffer, and 0.3μl lA taq (TaKaRa, Dalian Co., Ltd). The DNA templates were first subjected to an initial denaturation step for 2 min at 95°C. The subsequent cycles consisted of a 40 s denaturation step at 94°C, 40 s annealing step at 55°C, and 1.5 min extension step at 72°C. A final 7 min extension at 72°C was included after 30 cycles of PCR amplification. After purification the resultant PCR products were digested with Hinf-I for 3 h, then the digestion fragments of 16 S rDNA were resolved on native poly-acryl amide (6%) gels, which were stained with ethidium bromide and photographed. Related sequences were obtained from the Genbank database by using the BLAST search program. Alignment of sequences was carried out with CLUSTAL X 1.8 software. A phylogenetic tree was constructed by using the neighbor-joining method as implemented within the MEGA3.1.

Effect of different environmental factors

Decolorization of azo dye by isolates were carried out in Dye-STM with 100 mg l^{-1} K-2 BP, different salt concentration, different temperatures (20–40°C) and pH (5.0–10.0).

At the same time, the Dye-STM with cell-free and sterilized of isolates was conducted as the controls. To prevent possible contamination by oxygen during sampling, bottles were opened only once, and as many bottles were incubated as measurements were planned. The assays were performed in duplicate.

Analytical methods

Absorbance of the dye-containing solution was measured at their respective λmax values using an UV-visible spectrophotometer (JASCO, V-560, UV/VS spectrophotometer), and absorbance was proportional to concentration over the range 0–75 mg l^{-1} for K-2BP. The relationship between absorbance and concentration was unaffected by

pH in the range of 6–9 and NaCl concentration in the range of 0.5–150 g l^{-1}.

The cell concentration was measured by optical density at 660 nm.

Results and discussion

Phylogenetic position of isolates

To analyze the phylogenetic position, the 16 S rDNA sequence of strain GTW (1455 bp, DQ 279849) was deterimined. Figure 1 showed the phylogenetic relationship between the strain GTW and other related microorganisms found in the GenBank database. The homology assay result indicated that the strain GTW was in the phylogenetic branch of the *Halomonas*.

Halotolerance of isolates

One particular characteristic of the isolate is that it could grow on a medium containing 15% sodium chloride (w/v). In order to determine its halotolerant properties, the specific growth rate of the strain was estimated at different concentrations of sodium chloride. The strain GTW did not grow in the absence of sodium chloride or in the presence more than 25% sodium chloride, and exhibited the maximum growth rate at 10–15% of sodium chloride (Fig. 2). The results indicated that it is halophilic organisms that strictly require sodium chloride for the growth. This result indicated that strain GTW is a halotolerant organism that is capable of growing in a saline environment, and strictly requires sodium chloride for its growth.

Effect of carbon source on decolorization of azo dye

The strain GTW could grow well and completely decolourize K-2BP when yeast extract or peptone was present in the medium; however, glucose, glycerol, sucrose, lactose and starch resulted in lower rates of growth and decolourization of these dyes (data not shown). The other literatures also reported the maximum decolourization of azo dyes in presence of yeast extract by other bacteria (Hu 1998; Moosvi et al. 2005).

Effects of different factors on decolorization of azo dye

Some environmental factors (e.g. temperature, pH and carbon sources) were reported to affect decolorization activity of microorganisms (Pearcea et al. 2003). Effects of temperature and pH on decolorization were shown in Figs. 3 and 4, respectively. The results suggested that the optical range of temperature were 28–35°C. The percentage of decolorization reached the highest value (98%) at 30°C. It was obvious that the strain GTW did not show high decolorization percentage of reactive brilliant red K-2BP out of the optimal range of temperature. According to Fig. 4, the strain GTW could tolerate wider pH range (6.5–8.5). Thus, the optimal temperature and the appropriate pH range were 30°C, 6.5–8.5, respectively.

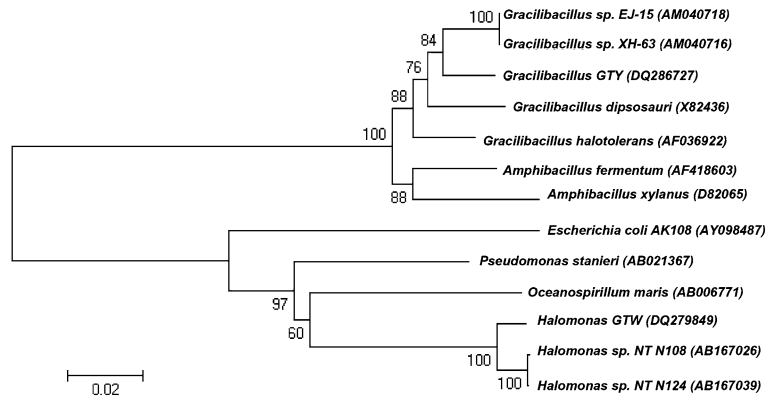

Fig. 1 Phylogenetic tree of halophilic bacterial strain GTW

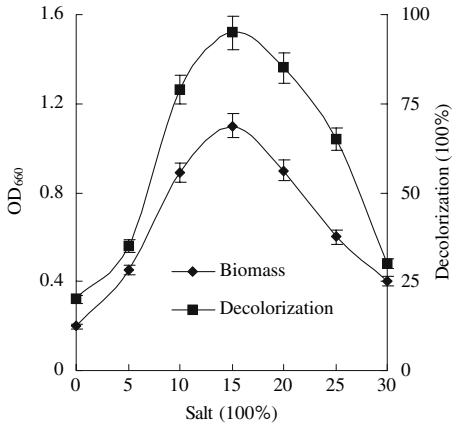

Fig. 2 Effect of NaCl on growth and decolorization of K-2BP by Halophilic sp. strain GTW

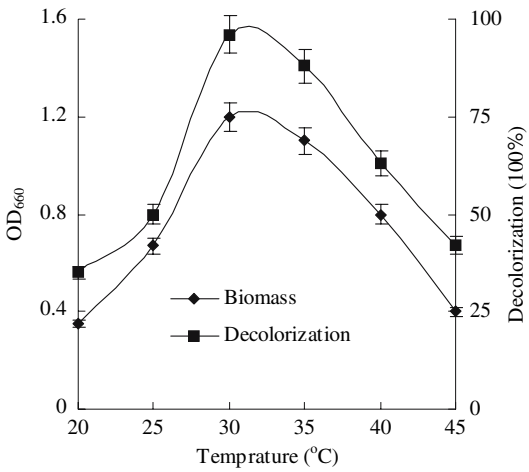

Fig. 3 Effect of temperature on growth and decolorization of K-2BP by Halophilic sp. strain GTW

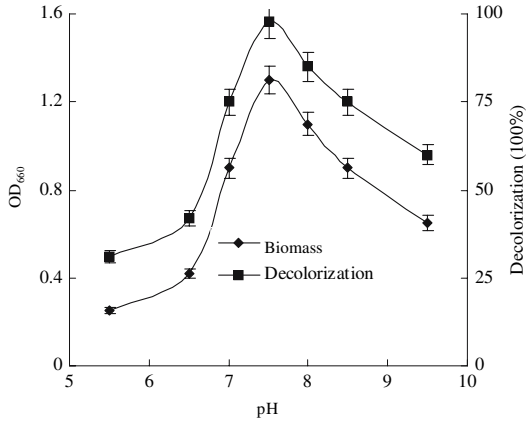

Fig. 4 Effect of pH on growth and decolorization of K-2BP by Halophilic sp. strain GTW

At the same time, the affection of different carbon sources on decolorization was studied. Addition of carbon sources (yeast extract and peptone) accelerated decolourization of the tested dyes; however, glucose, sucrose and glycerol resulted in lower rates of decolourization of these dyes. Yeast extract was selected as the carbon source in this study in light of the results data (data not shown).

Decolorization on different azo dyes

To demonstrate the general applicability of the strain GTW for the treatment of dye wastewater, various azo dyes were tested under anaerobic conditions at the 50 mg/l concentration. The results suggested that all selected azo dyes could be decolorizated by the strain GTW (Fig. 5),

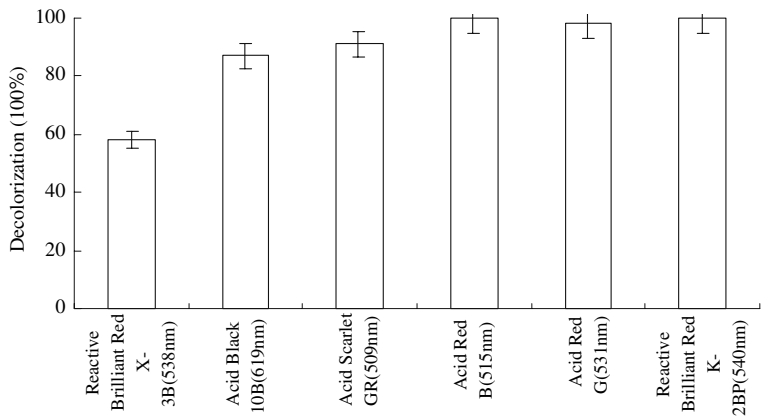

Fig. 5 Decolorization of various dyes in 24 h

which suggested that decolorization of azo dyes under anaerobic conditions was not a specific process and probably widespread (Seshadri et al. 1994; Bromley-challenor et al. 2000; Brown and De Vito 1993). The different decolorization of the tested azo dyes were affected by their molecular weights, substitution group of the dye molecules, and the intramolecular hydrogen bond between the azo and hydroxyl groups at the same time.

Other *Halomonas* spp. strains were isolated from the brine wastewater or other high salt environment, which could degrade phenol as the sole source of carbon and energy in a model industrial saline waste-water with NaCl-concentrations varying between 1 and 14% (w/v) NaCl (Hinteregger and Streichsbier 1997). A brown alga *Fucus evanescens* (Ivanova et al. 2002), and degraded hexahydro-1,3,5–trinitro-1,3,5-triazine (RDX) to N_2O and HCHO via the intermediary formation of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and methylenedinitramine. (Bhatt et al. 2005). However, to our knowledge strain GTW is the first halophilic bacteria strain shown to possess such a broad azo dye-decolorization range under anaerobic conditions.

Conclusions

The isolated strain GTW was a member of the genus *Halomonas* by 16 S rDNA sequence analysis and physic-biochemical tests, respectively. The number code of gene bank was DQ 279849.

Under high salt (NaCl) concentration, the strain GTW could decolorize many azo dyes, and the optimal conditions were 10–15% of sodium chloride, 30 °C and pH 6.5–8.5, respectively.

The results suggested that *Halomonas* sp. strain GTW holds potential for decolorization of colored effluents in high salt environments.

Acknowledgements This study was supported by the Cross-Century Talent Fund of National Education Committee of China.

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