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ORIGINAL PAPER

Dye-decolorization of a newly isolated strain *Bacillus amyloliquefaciens* W36

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Abstract

Dye-decolorization is one of the most important steps in dye-polluted wastewater treatment. The dye-decolorization bacteria were isolated from active sludge collected from wastewater treating pond of a dyeing and printing plant using serial dilution method. Among the 44 bacteria isolates from the active sludge, the strain *Bacillus amyloliquefaciens* W36 was found to have strong ability in dye-decolorization. The effects of carbon source, nitrogen sources, C/N, metal ions, temperature, pH, and rotation speed for dye-decolorization were investigated. The optimum decolorization conditions were that the strain was grown in enriched mineral salt medium (EMSM) using maltose 1 g/L, $(NH_4)_2SO_4$ 1 g/L as carbon and nitrogen source respectively, supplemented with 100 mg/L different dyes (pH 6.0), at 30 °C, 200 rpm from 48 to 96 h. The bacteria could aerobically decolorize dyes, such as Coomassie brilliant blue (95.42%), Bromcresol purple (93.34%), Congo red (72.37%) and Sarranine (61.7%), within 96 h. The dyes decolorization products were analyzed by ultra-violet and visible (UV–vis) spectroscopy before and after decolorization, which indicated that the four dyes were significantly degraded by the strain. The results indicated that the bacteria *Bacillus amyloliquefaciens* W36 could be used in dye-polluted wastewater treatment.

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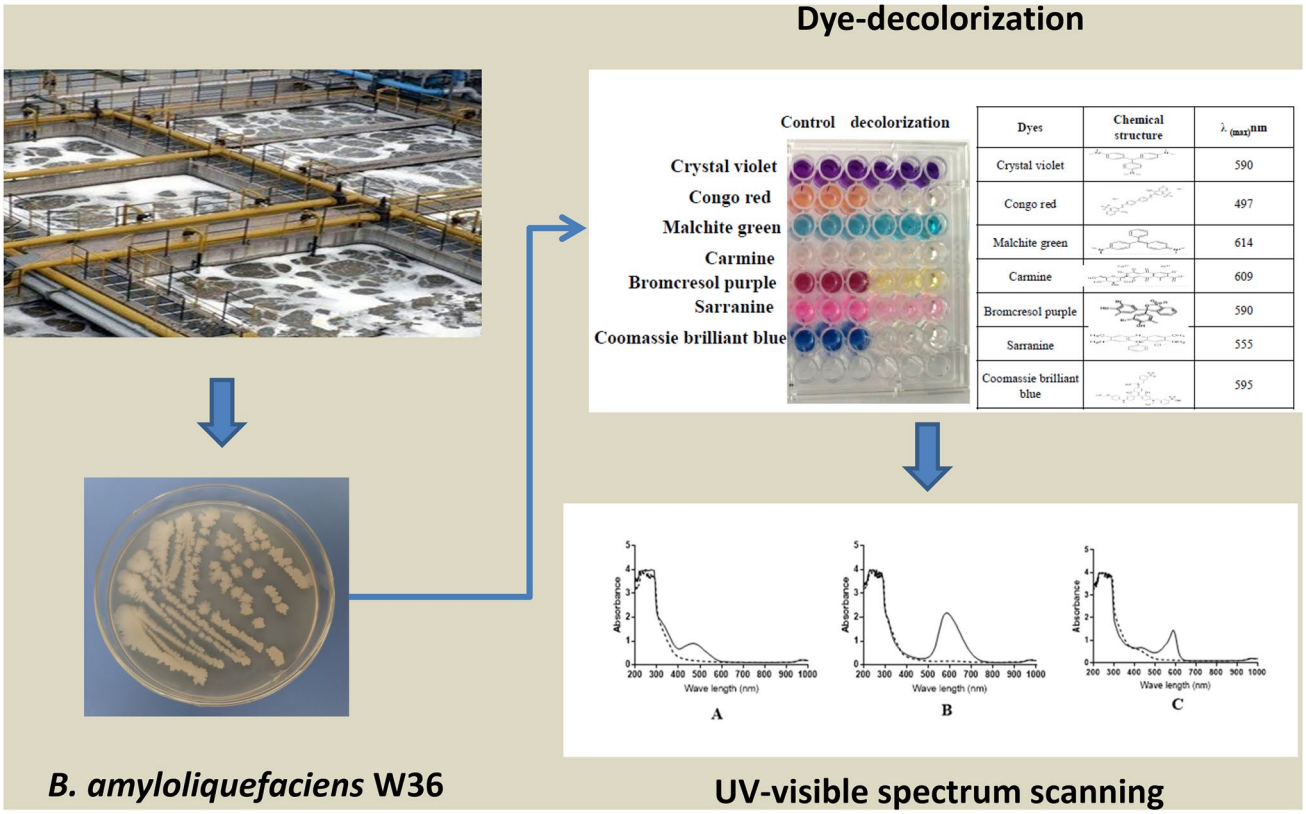
Graphic abstract



Keywords *Bacillus amyloliquefaciens* W36 · Commassie brilliant blue · Bromocresol purple · Congo red · Sarranine · Dye-decolorization

Introduction

Dyes are widely used in many industries, such as the textile, printing, food, pharmaceutical industry, and so on (Piaskowski et al. 2018). There are approximately 10,000 different dyes, which are classified according to their usage, such as direct dyes, reactive, disperse dyes, acid dyes, and cationic dyes, et al. Most of the dyes have complicated groups in structure, such as aromatic, azo, anthraquinone, triarylmethane or phthalocyanine, which are the toxic, carcinogenic and mutagenic chemicals to human or other creatures (Feng et al. 2012; Freeman 2013; Vikrant et al. 2018). These dyes are chemically synthesized. The synthetic dyes are highly stable to light, temperature, detergent and are very cheap and convenient in use (Vikrant et al. 2018). So they have been increasingly used in the textile and dyeing industries. Approximately 10–15% of used dyes would be discharged into the wastewaters in dyeing processes. However, these dyes are very hard to be degraded in the environment. The discharge of the dye-containing wastewater without well treatment will cause serious harm to the aquatic ecosystem and will become a carcinogenic source to humans (Schneider et al. 2004). As a result, how to treat the dye-polluted wastewater has raised great concern for the government and scientists.

China is one of the largest textile and dye-producing countries in the world. The annual dye production in China has reached 0.75 million tons since 2007, which accounted for 60% of world's dye production. In textile manufacturing and dyeing processes, if the dye-polluted wastewater was not well treated, it would cause serious pollution to water environments (Ren et al. 2013).

In general, dye-polluted wastewater is often treated by physical or chemical methods, such as coagulation, precipitation, adsorption, or physic-chemical methods by electrolysis or ozone oxidation method to destroy the dye molecules (Muhd Julkapli et al. 2014; Piaskowski et al. 2018; Vikrant et al. 2018). However, these methods are generally costly, inefficient, and of limited applicability in treating the dye effluent (Piaskowski et al. 2018; Vikrant et al. 2018). They produce some wastes, which are even harder to be degraded (Piaskowski et al. 2018). The

biological treating processes in wastewater purification are more attractive, because of their low cost. They produce less sludge, and are environmentally friendly (Vikrant et al. 2018). Many different aromatic or azo recalcitrant dyes could be degraded by some special microorganisms possessing the dye-degrading ability in the environment. Over the past decades, many microorganisms which were capable of degrading synthetic dyes had been found and studied, including fungi, yeasts, actinomycetes, and bacteria (Blanquez et al. 2019; Kaushik and Malik 2009; Pajot et al. 2011; Zablocka-Godlewska et al. 2018). Some fungi, especially white rot fungi, could produce ligninolytic enzyme, such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase and could be applied in the decolorization of dyes (Kaushik and Malik 2009; Rodriguez Couto 2009; Wesenberg et al. 2003). Some bacteria have been reported to have strong efficiency in dye-decolorization. The mechanism of dye-decolorization in microorganisms was supposed to be absorption, biodegradation or biotransformation (Kuhad et al. 2004). Different microorganisms have their own special way of decolorizing the various types of dyes. The growth speed of fungi is slow, which would decrease the efficiency in wastewater treatment (Fu and Viraraghavan 2001; Kaushik and Malik 2009; Piaskowski et al. 2018). So, the applications of fungal decolorization were limited (Kaushik and Malik 2009). Although many bacteria have been found to have the ability of dye-decolorization, most of the bacteria could only decolorize certain types of dyes (Cerboneschi et al. 2015; Lalnunhlimi and Krishnaswamy 2016; Modi et al. 2010). So, it is necessary to find more specific bacteria to decolorize various types of dye.

Our study aims to isolate some bacterial strains, which have strong abilities in dye-decolorization. A bacterium *B. amyloliquefaciens* W36 possessing the ability to decolorize dyes efficiently was chosen for further study and the factors affecting dye-decolorization were investigated.

Materials and methods

Chemicals and reagents

The chemicals of seven dyes Crystal violet, Congo red, Malachite green, Carmine, Bromocresol purple, Sarranine, and Coomassie brilliant blue were obtained from Parduk Company (Jinghua, Zhejiang, China). All the other chemicals used in this study were analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The enzyme and reagents used in PCR amplification were obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

Isolation and screening of the dye-decolorization strains

The strains were isolated from the active sludge, which was collected from the wastewater treating pond in Fenghuang Company, Meixinda or Dagong Textile Printing and dying Co., Ltd (Huzhou, Zhejiang, China). The enrichment culture was conducted by adding 1 g of active sludge into 100 mL of enriched mineral salt medium (EMSM) in a 250 mL Erlenmeyer flask and incubating in a shaker at 30 °C 120 rpm for 7 days. The mineral salt medium (EMSM) was consisted of (g/L): K_2HPO_4 1.0 g, KH_2PO_4 1.0 g, $MgSO_4$ 0.2 g, $FeSO_4$ 0.05 g, NH_4SO_4 1.0 g, glucose 1 g, yeast extract 2.5 g, sodium lignosulfonate 0.3 g, pH adjusted to 7.0. The strains were isolated by serial dilution of the cultural broth solution to 10^{-7}–10^{-9} and plating in EMSM solid medium. The plates were incubated at 30 °C for 2–3 days. The individual colony was picked up and streaked 2–3 times in EMSM to purify the strain. The strains were stored in the EMSM medium containing 15% (v/v) glycerol at − 80 °C.

Congo red has several aromatic rings and complicated structures and is one of the commonly used dyes for screening of decolorization strain (Ibrahim et al. 1996). The strains were screened by inoculating each strain to 10 mL EMSM liquid medium supplemented with Congo red (0.1 g/L) and incubated in a shaker at 30 °C 120 rpm for 7 days. Samples were taken from the culture broth and centrifuged at 12,000 rpm for 3 min at room temperature. 200 µL of supernatant was taken to measure the absorbance of Congo red at 497 nm using a microplate reader (ThermoFisher, USA). The strain with the highest decolorization rate was selected for further study.

Identification of the selected dye-decolorizing strain

The strain was grown in EMSM medium at 30 °C for 48 h. The morphological and physiological features of the strain were observed and tested by the method described by Buchanan et al. (1994). The genomic DNA of the strain was extracted by the method described previously by Steiner et al. (1995). The 16S rRNA gene was amplified with the primers 16S-F (5′-AGAGTTTGATCATGGCTCAG-3′) and 16S-R (5′-TACGGTTACCTTGTTACGACTT-3′). The PCR reaction was performed in 50 µL reaction system under the conditions, initial denaturation at 94 °C for 4 min, followed by denaturation at 94 °C for 30 s, annealing at 52 °C for 1 min, extension at 72 °C for 1 min 30 s, and a final extension at 72 °C for 10 min. The PCR product was purified and sequenced by Sangon Biotechnology Co., Ltd (Shanghai, China). The 16S rRNA sequence of the strain was submitted and blast in Genbank. The 16S rRNA sequences which were highly phylogenetically similar to the decolorization

strain were retrieved from Genbank. The phylogenetic tree was constructed using software MEGA V 7.0.26 through neighbor-Joining methods.

Effect of culture medium components on dye-decolorization

To investigate the effect of various carbon sources and nitrogen sources on dye-decolorization, 1% (w/v) different carbon sources (glucose, fructose, maltose, lactose, starch) and 1% (w/v) nitrogen sources $(NH_4)_2SO_4$, $NaNO_3$, NH_4Cl, urea, peptone, yeast extract, corn steep liquor, fish meal, soybean cake) were supplemented in EMSM medium, respectively. The strain was activated by inoculating the strain into 20 mL EMSM medium in a 100 mL Erlenmeyer flask and incubating at 30 °C shaker with a rotation speed of 120 rpm and culturing for 16–18 h until OD600 reached 0.8–1,0. Then 1 mL culture broth was inoculated into 50 mL EMSM medium containing 1%(w/v) of different carbon sources or nitrogen sources and 0.1 g/L Congo red in a 250 mL Erlenmeyer flask. The strain was cultured in a shaker at 30 °C for 7 days with a shaking speed 200 rpm. Samples were taken every 2 days from the cultural broth and used for test cell density and dye concentration.

To study the effect of carbon source and nitrogen sources (C/N) ratio on dye-decolorization, the best carbon source and nitrogen source were selected. The different C/N ratio was set as (1:1, 2:1, 3:1 4:1, w/w) in the EMSM medium. The strain was cultured with the same conditions as above.

Metal ions are often activators or inhibitors to the enzyme involved in dye-decolorization (Ibrahim et al. 1996). To check the effect of metal ions on dye-decolorization, 5 mM different metal ions of sulphate (Cu^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Co^{+}, Na^{+}, Fe^{2+}, K^{+}) were supplemented in the EMSM medium containing 0.1 g/L Congo red, with the best carbon, nitrogen source and C/N for dye-decolorization. The strain was cultured with the same conditions as above.

Effect of culture conditions on dye-decolorization rate

The strain *B. amyloliquefaciens* W36 was grown in 20 mL EMSM medium in a 100 mL Erlenmeyer flask at 30 °C and 120 rpm for 16–18 h as inoculums. All the decolorization experiments were performed in a 250 mL Erlenmeyer flask. 1 mL of culture broth was inoculated into 50 mL EMSM medium containing Congo red 0.1% (w/v) and incubated in a shaker at 30 °C 120 rpm for 7 days. Effect of different factors on the decolorization of Congo red was investigated via a single factor experiment, i.e. changing the factors once at a time and keeping other parameters as constants. The factors were temperature (10, 25, 30, 35 °C), pH (5.0, 6.0, 7.0, 8.0, 9.0, 10.0) and shaking speed (100, 150, 200, 250 rpm). When investigating the factor temperature, the pH of EMSM medium was adjusted to 7.0. When investigating pH, the temperature for dye-decolorization was set at the optimum condition. When investigating rotation speed, the best temperature and pH for dye-decolorization were fixed at the optimum condition. The samples were taken from the culture broth after cultivation for 96 h. The cell density was measured by a microplate reader (Thermofisher, USA) at 600 nm. The dye concentration was assayed at the maximum absorbance of the dyes using the same microplate reader. The cultural medium without inoculation was used as blank. All assays were performed in triplicate.

The dye-decolorizing ability of the strain

To detect the decolorizing ability of the strain, different 0.1 g/L dyes (Crystal violet, Congo red, Malachite green, Carmine, Bromocresol purple, Sarranine, Coomassie brilliant blue) was supplemented in EMSM medium with the optimum carbon, nitrogen source, metal ions, and C/N. The strain was cultured with the same conditions as above.

Analysis methods

The cell density and dye concentration were measured by a microplate reader (Thermofisher, USA) at 600 nm or the maximum absorbance of each dye, respectively. The maximum absorbance of the dyes Crystal violet (λ_{max} 590 nm), Congo red (λ_{max} 497 nm), Malachite green (λ_{max} 614 nm), Carmine (λ_{max} 609 nm), Bromocresol purple (λ_{max} 590 nm), Sarranine (λ_{max} 555 nm), Coomassie brilliant blue (λ_{max} 595 nm) were obtained by scanning the 0.1 g/L dye-containing EMSM medium. All assays were performed in triplicate. The cultural medium without inoculation was used as blank. The decolorized product was scanned with the microplate reader (Thermofisher, USA) in the wavelength range of 200 to 1000 nm.

The decolorization efficiency was calculated by the formula. Decolorization rate $(\%) = (A0 - A)/A0 \times 100$, where A0 is the initial absorbance and A is the absorbance of the medium after decolorization with the strain.

Results

Isolation and identification of the dye-decolorizing strain

After several times of isolation from the active sludge samples, 44 dye-decolorization strains were obtained. The strain with the highest decolorization rate (56.13%) to Congo red was selected for further study. The colonies of

the strain were flat-shaped and bulged in the middle, light yellow, rough on the surface and irregular on the edge.

The 16S rRNA gene of the strain was amplified by PCR. The sequence of it was deposited in GenBank (accession number SUB6817070 *Bacillus* MN922613). The nucleotide alignment of 16S rRNA sequence of the strain with Genbank showed that the strain exhibited 100% homology to *Bacillus amyloliquefaciens*. The evolutionary relationship analysis from the phylogenetic tree indicated that the strain has the closest relationship with the strain *Bacillus amyloliquefaciens* strain md1-41 (Fig. 1). Therefore, it was identified as *Bacillus amyloliquefaciens* W36.

Effect of culture medium on dye-decolorizing

The effect of culture medium on dye-decolorizing was investigated (Fig. 2). Carbon sources affect the dye-decolorization of the strain significantly. As shown in Fig. 2a, maltose is the best carbon source for dye-decolorization. The dye-decolorization efficiency of the strain is 80.66% when maltose was used as the carbon source. It is much higher than that of other carbon sources in culture medium.

To investigate the effect of nitrogen source on decolorization rate, various organic and inorganic nitrogen sources were added into the culture medium. As it is shown in Fig. 2b, the decolorization rate was greatly affected by the

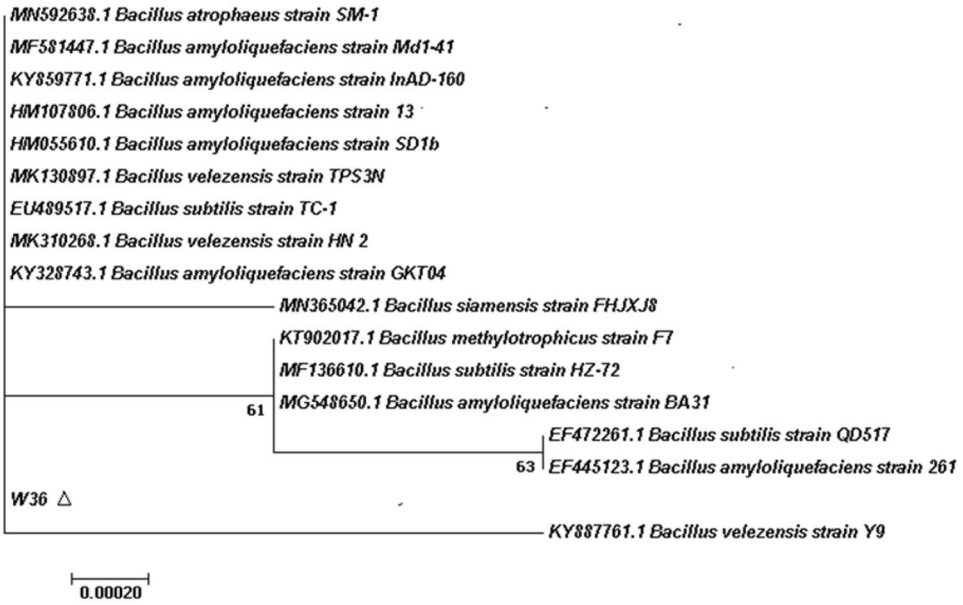


Fig. 1 Phylogenetic tree of *B. amyloliquefaciens* W36. The GenBank accession number was shown in front of each species of the microorganism. Bootstrap values were exhibited at each branch. Scale bar indicated 0.0002 phylogenetic distances

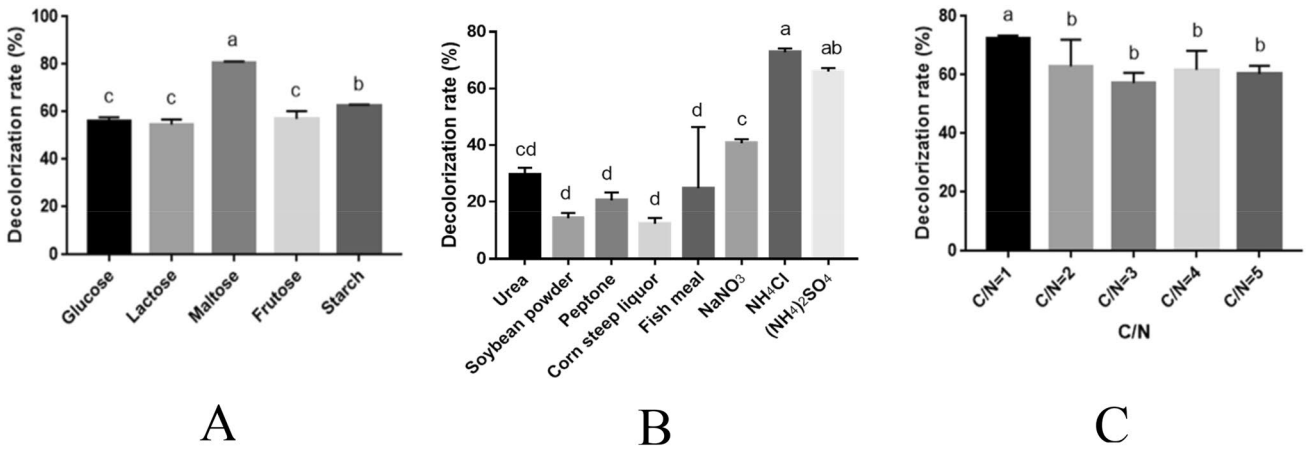


Fig. 2 Effect of cultural medium on dye-decolorization rate. **a**. Carbon sources; **b** Nitrogen sources; **c** C/N. The strain *B. amyloliquefaciens* W36 was cultured in EMSM medium supplemented with 0.1% (w/v) Congo red and different C/N at 30 °C 200 rpm for 96 h. Means and standard error of mean were shown in the figures (mean ± SEM, n = 3). Lower case letters indicate the variation between different groups, as analyzed by one-way ANOVA ($P < 0.05$)

addition of various nitrogen sources. NH_4Cl and $(NH_4)_2SO_4$ were the best nitrogen sources for dye-decolorization. The decolorization efficiency was achieved at 72.80% or 65.97%, respectively.

C/N is also an important factor affecting decolorization. It could be shown from Fig. 2c that the highest decolorization rate (72.46%) was achieved when C/N was set as 1:1. With the increase of the ratio of C/N, the decolorization rate was decreased slightly.

The effect of metal ions on the decolorization rate is shown in (Fig. 3). The best metal ions for dye-decolorization for this strain are the addition of 5 mM K^+ or Na^+ to the culture medium and the decolorizaition efficiency was reached at 75.41% or 77.01%, respectively. The addition of Co^+ in the cultural medium reduced the dye-decolorization efficiency, which might be because Co^+ is harmful to the cell growth or metabolism. Metal ions are the activator or inhibitor of the key enzymes involving in the dye-decolorization, which will affect the enzyme activities (Ibrahim et al. 1996).

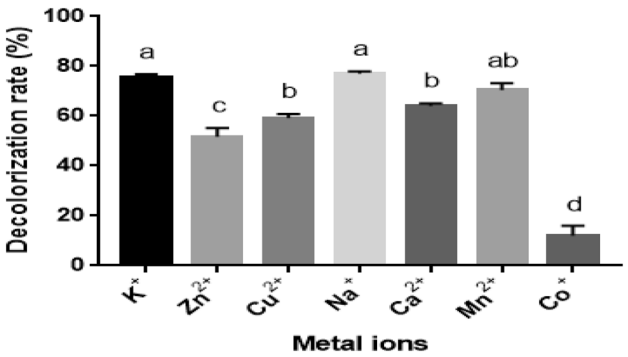


Fig. 3 Effect of different metal ions on dye-decolorization rate. The strain *B. amyloliquefaciens* W36 was cultured in EMSM medium supplemented with 0.1% (w/v) Congo red and different metal ions at 30 °C 200 rpm for 96 h. Means and standard error of mean were shown in the figures (mean ± SEM, n = 3). Lower case letters indicate the variation between different groups, as analyzed by one-way ANOVA ($P < 0.05$)

Effect of culture conditions on dye-decolorizing

The effects of cultural conditions on the decolorization rate of *B. amyloliquefaciens* W36 were shown in (Fig. 4). It could be inferred from the results that the decolorization rate increased as temperature increased (Fig. 4a). The dye-decolorization efficiency was achieved 51.42% at the optimum temperature 30 °C. Most microorganisms have an optimum temperature range to grow. The strain can grow better and utilize the substrate at the optimum temperature. The optimum pH for decolorization and cell growth was pH 6.0. The cell growth and decolorization rate decreased as the pH increased (Fig. 4b). pH is also important for bacterial growth. The decolorization rate increased with the rotation speed was increased. The cell growth decreased slightly when the rotation speed increased from 100 to 200 rpm, while the cell growth increased at the rotation speed of 250 rpm (Fig. 4c). It is indicated that the strain is aerobic in decolorization.

The decolorization ability assay

The decolorization ability on 7 different dyes by the strain *B. amyloliquefaciens* W36 was studied (Fig. 5). The strain could decolorize Bromocresol purple and Coomassie brilliant blue efficiently. The decolorization rate could achieve 95.42% and 93.34%, respectively. 72.37% of Congo red could be decolorized by this strain. However, it could not decolorize Crystal violet and Malachite green. It could decolorize Sarranine (61.07%) and Carmine (37.08%).

The dye-decolorization effects of the four dyes (Congo red, Bromocresol purple, Coomassie brilliant blue, and

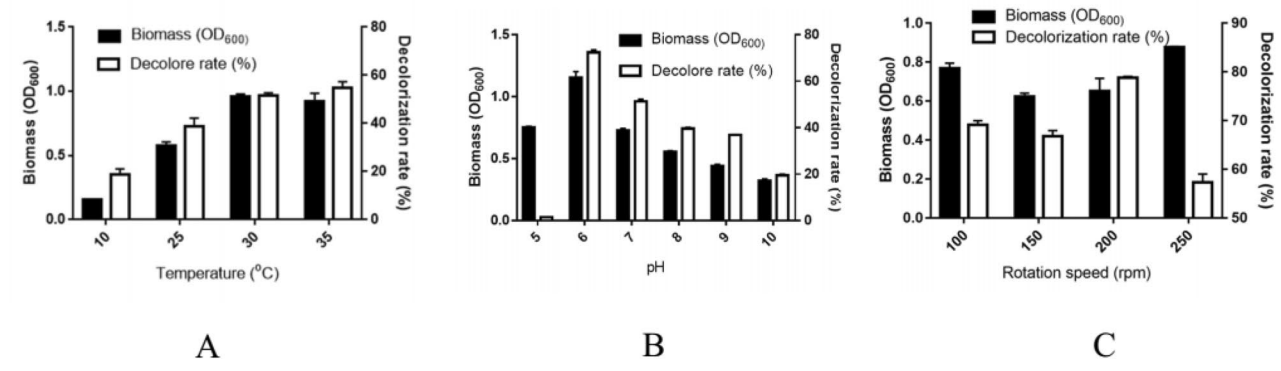


Fig. 4 Effect of cultural conditions on cell growth and dye-decolorization rate. **a** Temperature; **b** pH; **c** Rotation speed. The strain *B. amyloliquefaciens* W36 was cultured in EMSM medium supplemented with 0.1% (w/v) Congo red at different rotation speed (100–250 rpm) for 96 h. Means and standard error of mean were shown in the figures (mean ± SEM, n = 3)

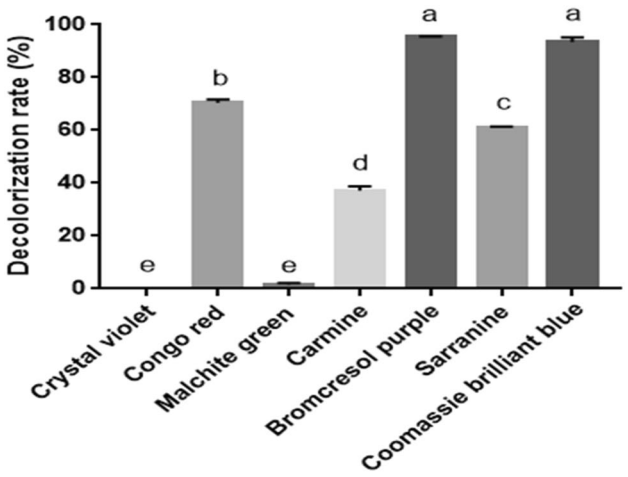


Fig. 5 The decolorization ability of the strain *B. amyloliquefaciens* W36. The strain *B. amyloliquefaciens* W36 was cultured in EMSM medium supplemented with 0.1% (w/v) different dyes at 30 °C 200 rpm for 96 h. Lower case letters indicate the variation between different groups, as analyzed by one-way ANOVA ($P < 0.05$)

Sarranine) were further studied. Results showed that the strain could decolorize Bromocresol purple and Coomassie brilliant blue within 48–96 h, decolorize most of Congo red and Sarranine after 72–96 h. The strain grew rapidly within 24 h supplemented with the dyes Congo red and Bromocresol purple in the culture medium, while it grew slowly before 48 h and rapidly after 48 h with dyes Coomassie brilliant blue and Sarranine in the culture medium. The four dyes did not affect the strain growth (Fig. 6). The decolorization results were clearly shown in Fig. 7.

As shown in Fig. 8, the peak absorbance of dyes almost decreased completely after 3 days of decolorization. The cell mat showed the same color as the dyes, which indicated the decolorization was also partly due to being absorbed by the strain.

Discussion

Some fungi have been found to have strong ability in dye-decolorization due to dye-biosorption or biodegradation (Fu and Viraraghavan 2001; Murugesan and Kalaichelvan 2003). However, fungi grow much slower than bacteria and need

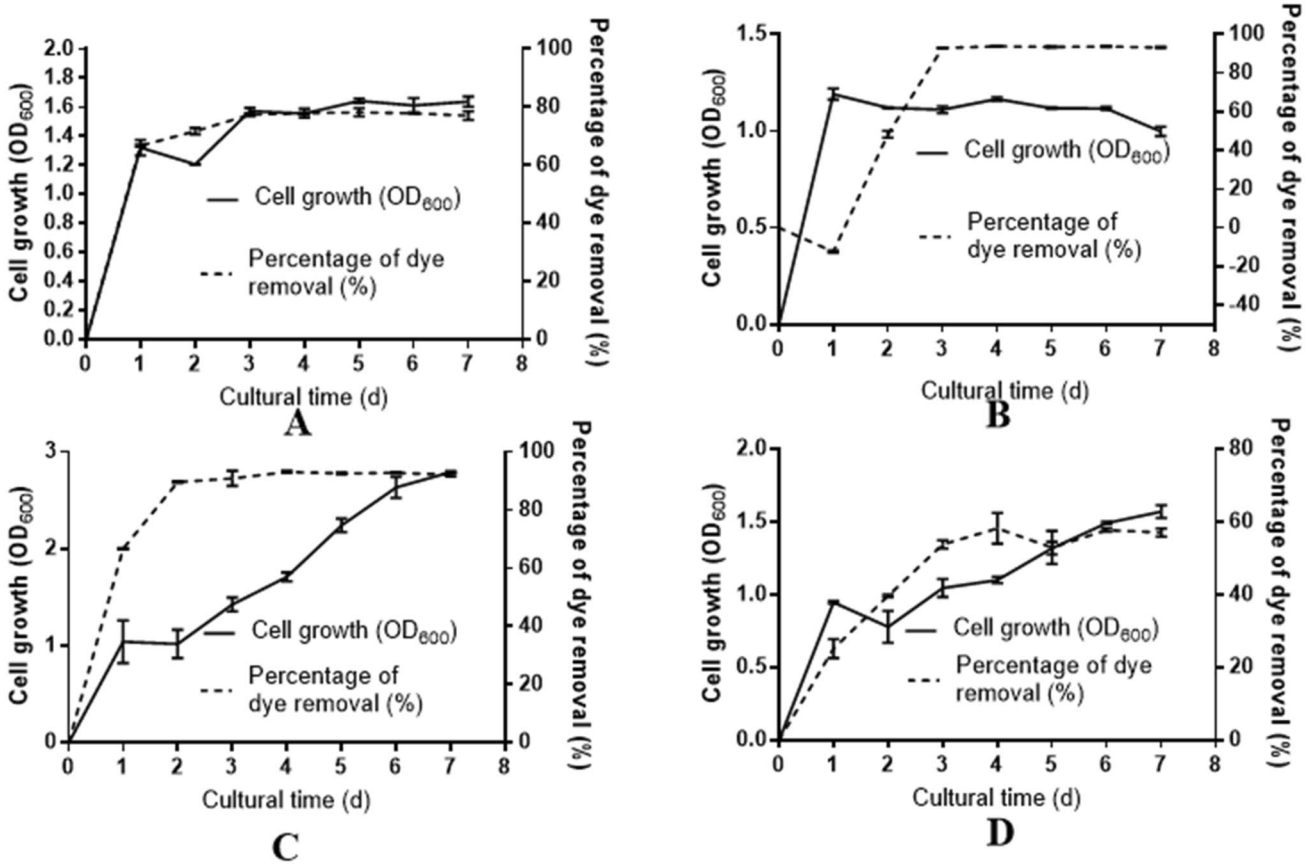
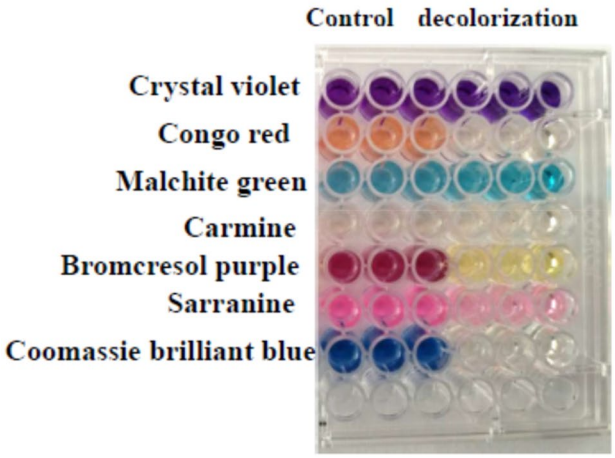


Fig. 6 The dye-decolorization of *B. amyloliquefaciens* W36 to different dyes. Solid line indicates cell growth (OD600), dash line indicates decolorization rate (%). **a** Congo red; **b** Bromocresol purple; **c** Coomassie brilliant blue; **d** Sarranine



Dyes	Chemical structure	$\lambda_{(max)}$nm
Crystal violet		590
Congo red		497
Malchite green		614
Carmine		609
Bromcresol purple		590
Sarranine		555
Coomassie brilliant blue		595

Fig. 7 The photograph of the strain decolorization of different dyes. The strain was cultured on EMSM medium in addition to different dyes at 30 °C for 96 h in 200 rpm shaker

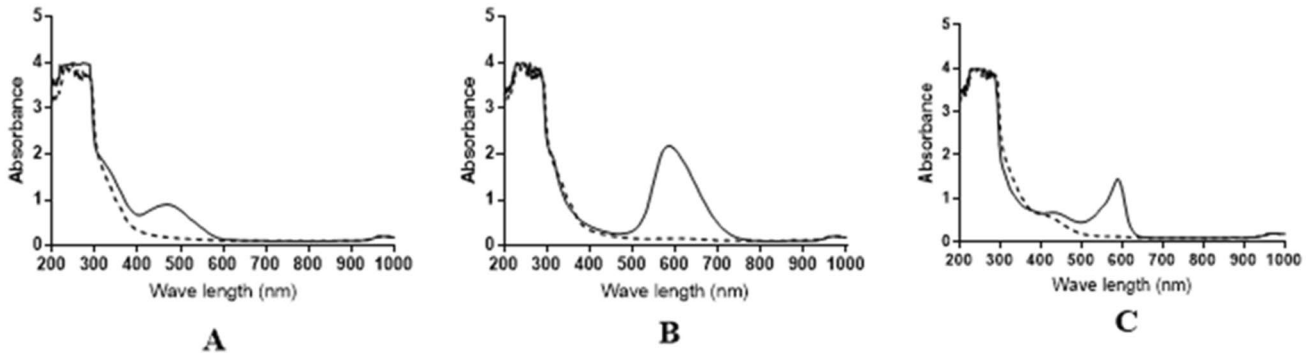


Fig. 8 UV–visible spectrum scanning the dye before and after decolorization with *B. amyloliquefaciens* W36 in EMSM medium containing different dyes at 30 °C 200 rpm for 96 h. Solid line (Control), Dash line (Experimental group). **a** Congo Red; **b** Coomassie brilliant blue; **c** Bromocresol purple

more time in dye-decolorization (Vikrant et al. 2018). In this study, we found *B. amyloliquefaciens* W36 could decolorize four different dyes Coomassie brilliant blue (95.42%), Bromcresol purple (93.34%), Congo red (72.37%) and Sarranine efficiently within 48–96 h, which is faster than most of fungi. The dye-decolorization efficiency was compared with other strains (Table 1), *B. amyloliquefaciens* W36 showed good efficiency in dye decolorization in the optimum conditions.

Dye-decolorization efficiency was greatly affected by cultural medium and cultural conditions. Cultural medium affects strain growth, metabolism, and enzyme production, which has a significant influence on dye-decolorization. The most preferred carbon resource for the strain *B. amyloliquefaciens* W36 growth and decolorization was maltose. However, the favorite carbon source for other strains was glucose or sucrose (Holkar et al. 2014; Lalnunhlimi and Krishnaswamy 2016; Wang et al. 2009). Because the metabolism of dye-decolorization pathway may be different, the favorite carbon source may be various from different strains. Nitrogen sources are also important to strain growth and dye-decolorization. In this study, NH_4Cl and $(NH_4)_2SO_4$ are found to be the best nitrogen sources for strain growth and decolorization. While the best nitrogen sources for decolorization were yeast extract or peptone in some other strains (Chen et al. 2003; Modi et al. 2010). NH_4Cl and $(NH_4)_2SO_4$ are inorganic nitrogen sources, which have been commonly used in culture medium to cultivate strains and they are easy to be utilized to grown cells or produce some metabolites. Carbon sources are usually used to grow cells in strain cultivation. Addition of too much of carbon source in culture medium may not benefit bacteria to decolorization. The results are in accordance with previous reports that a high concentration of carbon sources in the culture medium will inhibit the decolorization of some bacteria (Chen et al. 2003). In our study, we found the addition of 5 mM K^+ or Na^+

Table 1 Comparison of the decolorization efficiency and decolorization conditions of different strains

Strains	Decolorization dyes	Decolorization conditions	Time	Decolorization efficiency	References
Geobacter metallireducens	Azo dyes including acid orange 7 (AO7), acid red 27, acid red 73 (AR73), reactive red 120 (RR120), direct blue 71 (DB71), and acid orange 52 (AO52)	The strain could anaerobically decolorize several aze dyes in bicarbonate-buffered medium, in addition of low concentrations of humic acid (1–100 mg/L) or 2-hydroxy–1,4-naphthoquinone (0.5–50 μM) at 30 °C	40 h	66.3% ± 2.6–93.7 ± 2.1%	Kolekar and Kodam (2012)
Bacillus cereus M1 and M6	Reactive Red195 (RR195), Reactive Black5 (RB5), Reactive Black Reactive Yellow145 (RY145), and Reactive Black	The strain were grown in nutrient broth and incubated with different dyes at 37 °C in static conditions	72 h	48.3–84.3%	Modi et al. (2010)
Scheffersomyces spartinae TLHS-SF1	Acid Scarlet 3R,Acid Red B, Acid Orange II, Acid Scarlet GR, Reactive Brilliant, Red K-2G	The strain was grown in the cultural medium supplemented with sucrose 2 g/L, $(NH_4)_2SO_4$ 0.6 g/L, yeast extract 0.08 g/L, NaCl ≦30 g/L and 20 mg/L different dyes, pH 5.0–6.0 at 30 °C 160 r/min	12–24 h	96.14–98.14%	Tan et al. (2016)
Alkaliphilic bacterial consortium	Direct Blue 151 (DB 151) and Direct Red 31 (DR 31)	The bacterial consortium was enriched in MSM (pH 9.5) amended with 100 mg/L of DB 151 and DR 31, at 37 °C, 200 rpm	5 days	97.57% and 95.25% respectively	Lalnunhlimi and Krishnaswamy (2016)
B. amyloliquefaciens W36	Coomassie brilliant blue, Bromocresol purple, Congo red and Sarranine	The strain was grown in EMSM using maltose 1 g/L, $(NH_4)_2SO_4$ 1 g/L as carbon and nitrogen source, supplemented with 100 mg/L different dyes (pH 6.0), at 30 °C, 200 rpm	48–96 h	61.07–95.42%	This study

to the culture medium could enhance decolorization efficiency. Metal ions have often been used as an activator for enzymes or been used to adjust osmotic pressure in the cell. The metal ions requirements for decolorization are different for different microorganisms (Galai et al. 2009; Shankar and Shikha 2015; Tan et al. 2009).

The dye-decolorization was affected by temperature, pH, and oxygen significantly. In this study, we found that the decolorization rate increased with temperature increase. The results are similar to most of the bacterial decolorization (Karim et al. 2018; Kolekar and Kodam 2012; Lalnunhlimi and Krishnaswamy 2016). pH plays an important role in cell growth and dye-decolorization. The strain *B. amyloliquefaciens* W36 grows and decolorizes at a narrow pH range. The results are in accordance with most decolorizing bacteria that have a narrow pH range in decolorization (Lalnunhlimi and Krishnaswamy 2016; Tan et al. 2016). While in some other strains, the suitable pH ranges for dye-decolorization were broad (Anjaneya et al. 2011; Chen et al. 2003; Ren et al. 2006). The strain *B. amyloliquefaciens* W36 grows and decolorizes better in aerobic conditions. It needs oxygen in dye-decolorization. The sample was taken from the oxygen- exposed pond of wastewater treatment, so it is highly possible that the strain is an aerobic strain. Many strains could decolorize dyes under the aerobic condition (Tan et al. 2016, 2013).

In our study, we found the cell mat showed the same color as the dyes, while the supernatant showed very less color in decolorization. It indicated the strain could absorb or biodegrade the dyes in the decolorization process. It has been reported that decolorization of dyes by bacteria could due to absorption or biodegradation (Keharia and Madamwar 2003; Piaskowski et al. 2018). The plasmid was extracted from the strain. However, no plasmid was extracted from it which indicated that the strain has no biodegradation of plasmid in its cell (data not shown). No laccase activity was detected from the strain (data not shown). The dye-biodegradation mechanism was still under investigation.

The dye-decolorization is the key step in dye-polluted water treatment. The strain *B. amyloliquefaciens* W36 could decolorize various dyes efficiently under optimized conditions. Compared with the decolorization strain discovered in this species, *B. amyloliquefaciens* W36 could decolorize four different dyes efficiently and do not produce laccase. It indicated that the decolorization mechanism was different from the reported strains (Lončar et al. 2013; Wang et al. 2017). Because of the complicate dye structures, each of the bacteria cannot decolorize all types of dyes (Kuhad et al. 2004). We need to find more and more strains with strong ability to be used in dye polluted wastewater treatment. The strain may have practical application for the biological purification of dye-containing wastewater.

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Author contributions YL conceived and designed research and wrote the manuscript. ZYS, XPR and JNZ conducted experiments and analyzed data. WQ reviewed and revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declared that have no conflict of interest.

Ethical approval The authors do not use human tissues or animals to conduct the experiment.

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