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ORIGINAL ARTICLE

Decolorization and biodegradation of triphenylmethane dyes by a novel *Rhodococcus qingshengii* JB301 isolated from sawdust

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Abstract A sawdust sample collected from a wet environment was used to screen for crystal violet-decolorizing and methyl violet-decolorizing bacterial strains. A strain with degrading and decolorizing abilities was isolated and identified as *Rhodococcus qingshengii* JB301. The decolorization of crystal violet and methyl violet was improved by optimizing culture conditions. Based on liquid chromatography–mass spectrometry (LC-MS) analysis, similar degradation products of crystal violet and methyl violet were detected, while [N,N-dimethylaminophenyl] [aminophenyl] benzophenone or [N-methylaminophenyl] [N-methylaminophenyl] benzophenone was different from previous reports. A common degradation pathway was established to demonstrate the mechanism of crystal violet and methyl violet decolorization. It was found that lignin peroxidase activity was greatly enhanced after addition of crystal violet (by 514 %) and methyl violet (by 698 %) at 12 h, while NADH-DCIP (dichlorophenolindophenol) reductase activity was inhibited by the dyes. The growth of *Triticum aestivum* was inhibited by both dyes and the toxicity of the dyes was weakened after the degradation by *R. qingshengii* JB301.

Keywords Biodegradation · Chromatography · Fermentation · Enzyme activity · *Rhodococcus qingshengii* · Triphenylmethane dyes

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Introduction

Triphenylmethane dyes, including crystal violet, methyl violet, malachite green and brilliant green, are widely applied in several industries such as textiles, cosmetics, paper and as biological stains (like gram stain) (Beveridge 2001; Parshetti et al. 2006; Nohynek et al. 2010; Kumar et al. 2011). Improper disposal of dye effluents in lakes and rivers leads to reduced sunlight penetration, which in turn decreases both photosynthetic activity and dissolved oxygen concentration. Due to hazardous products released during decomposition, dye pollutants from these industries had toxic effects on flora and fauna (Kumar et al. 2005; Soloman et al. 2010). In addition, human exposure to these dyes may cause a severe risk due to their toxicological and carcinogenic properties on the immune system and respiratory system (Fessard et al. 1999; Kumar et al. 2005; Culp et al. 2006).

Several physical and chemical methods, such as flocculation, coagulation, adsorption, membrane filtration, precipitation and irradiation/ozonization, are applied to treat wastewater containing colored effluents (Franciscon et al. 2010). However, the main drawbacks of physical and chemical methods, such as the high cost, inability to remove recalcitrant dyes and production of large amounts of sludge, limit their applications (Robinson et al. 2001; Mittal et al. 2005; Khataee et al. 2009). Much attention has been devoted to the microbial decolorization and degradation of dyes because of the advantages of being inexpensive and producing less amounts of

toxic sludge. It has been reported that a variety of dyes can be biodegraded by a wide range of microorganisms, including fungi (Chander and Arora 2007), bacteria (Manal et al. 2005), yeasts (Jadhav and Govindwar 2006), actinomycetes (Yatome et al. 1993) and algae (Khataee et al. 2011).

Rhodococcus sp. bacteria are widely distributed in soil and possess the ability to degrade a range of compounds. Due to their metabolic properties, *Rhodococcus* species have been employed for the degradation of toxic compounds and chemicals (Behki et al. 1993; Bell et al. 1998; Dean-Ross et al. 2001), production of enzymes (Kamble et al. 2010; de las Heras et al. 2011) and the synthesis of bio-surfactant and flocculant (Marques et al. 2009; Chang et al. 2009). The degradation products and the pathway of dyes degradation have been analyzed in many studies (Yatome et al. 1993; Chen et al. 2008, 2010; Kumar et al. 2011; Parshetti et al. 2011). However, no intensive work has been carried out to investigate the possible mechanism of methyl violet and crystal violet degradation by *Rhodococcus* sp. In the present study, a dye decolorizing bacterial strain has been isolated from sawdust and identified as *Rhodococcus qingshengii* JB301. The decolorization ability of the isolated strain was studied under different culture conditions using crystal violet and methyl violet. The phytotoxic properties and the degradation products formed during the dye degradation were analyzed.

Materials and methods

Dyes and chemicals

Crystal violet and methyl violet used in this study were purchased from Sinopharm Chemical Reagent Co. (Beijing, China). Azure B (3-methylamino-7-dimethylaminophenothiazin-5-ium chloride) and ABTS [2,2'-azino-di-(3-ethyl-benzothiazolin-sulphonate)] were obtained from Shanghai Sangon Biotechnology (Shanghai, China).

Screening for microorganism and culture conditions

A sawdust sample collected from a wet environment (the riverside of Taihu Lake, Wuxi, China) was screened for dye-decolorizing bacterial strains. The sample was cultured with sterilized saline water and the culture broth was spread on an aniline blue agar plate. The culture broth was composed of (g/l): peptone 10, yeast extract 5, and NaCl 5 (pH 7.0). The aniline blue agar plates contained (g/l): peptone 10, beef extract 3, NaCl 5, aniline blue 1, and agar 20 (pH 7.0). Colonies producing transparent circles on the plate were isolated and inoculated into Luria-Bertani (LB) medium at 30 °C and 150 rpm. Twenty-five milligrams of crystal violet and methyl violet per liter were separately added to the culture broth after 24 h cultivation and the decolorizing ability of the isolated strain was measured. After the screening, one strain showing the greatest ability of decolorization was identified as *R. qingshengii* JB301 and preserved for further decolorization analysis.

16S rDNA sequencing

Fragments of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) as described by Weisburg et al. (1991). The primers used for amplifications were:

5′-AGAGTTTGATCCTGGCTCAG-3′ (primer F) and
5′-CGGTTACCTTGTTACGACTT-3′ (primer R).

The amplified 16S rRNA gene was sequenced with a Dye Terminator Cycle-sequencing FS Ready Reaction kit and a model ABI 3130 automatic DNA sequencer (Applied Biosystems) by Shensushengwu Co., Ltd (Shanghai, China). A homology search was performed with the GenBank database to determine the strain species.

Decolorization assay

In order to evaluate the decolorization ability of the isolated strain, 25 mg/l of crystal violet and methyl violet were added to the culture medium after 24 h incubation. The repetitive decolorization capacity of the cells was determined by consecutive addition of 5 mg/l crystal violet and 5 mg/l methyl violet to the culture broth separately. The culture broth was withdrawn at different time intervals and treated as previously described to measure the decolorization of crystal violet and methyl violet (Manal et al. 2005; Jadhav et al. 2008; Yang et al. 2011a, b). The control experiment was the same condition of the two dyes decolorization without the inoculation. Each experiment was performed in triplicates. The results were shown as the averages of three replicates, with error bars indicating the standard deviation.

Effect of culture conditions on decolorization

The decolorization of the two dyes was evaluated under different conditions, including pH (4 - 10), various temperatures (20 - 45 °C), different inoculation sizes of 0.1 ~ 10 % (volume per volume), and static and shaking (150 rpm) conditions. The concentrations of crystal violet and methyl violet were 15 mg/l each.

In order to check the effect of dye concentration on decolorization, 15, 30, 50, 100, 150 and 200 mg/l of crystal violet and methyl violet were separately added to the culture medium. The effect of dye (15 mg/l crystal violet and 15 mg/l methyl violet) addition time on decolorization was studied after incubation for 12, 24, 36, 48 and 60 h.

Enzyme activity assay

Rhodococcus qingshengii JB301 was grown in LB medium for 24 h and cells were collected by centrifugation at 8,000 rpm for 20 min. The cell precipitate was dissolved in 50 mM potassium phosphate buffer (pH 7.4) for sonication (sonics-vibracell ultrasonic processor). The sonifier output was kept at 60 A with eight strokes, 30 s each, at 2 min intervals. The temperature was maintained at 4 °C and the cell extract was used as an enzyme source without centrifugation (Parshetti et al. 2009).

The cell extract obtained by sonication was used to determine the activity of intracellular enzymes. The activity of lignin peroxidase (LiP) was measured using veratryl alcohol as the substrate at 300 nm (Tien and Kirk 1984). Laccase activity was determined spectrophotometrically at 420 nm using 10 % ABTS as the substrate in 0.1 M acetate buffer (pH 4.9) (Kalyani et al. 2008). Tyrosinase activity was determined at 495 nm in a mixture containing 0.01 % catechol with 0.1 M phosphate buffer (pH 7.4) (Kalyani et al. 2008). NADH-DCIP reductase activity was measured at 595 nm in the 5 ml reaction mixture contained 50 μM DCIP, 50 μM NADH, 50 mM potassium phosphate (pH 7.4) and 0.1 ml appropriately diluted crude culture broth with the molar extinction coefficient of 19 mM^{-1} cm^{-1} (Salokhe and Govindwar 1999). The protein content in the cell extract was measured as described by Bradford (1976).

Decolorization and biodegradation analysis

The culture broth from crystal violet and methyl violet decolorization was used to obtain degradation products. The supernatant was treated as previously described and the crystallized degradation products were dissolved for further determination by LC-MS (Jadhav et al. 2008). We used a ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC–Q-TOF MS) system equipped with a Waters Acquity SDS pump connected to a Waters Acquity PDA detector, Sunfire C18 column (150 mm×2.1 mm, 5 μm), and time-of-flight mass spectrometer with electrospray ionization source. Solvent A was acetonitrile and Solvent B was 20 mM ammonium acetate. The proportion of A was increased linearly from 30 % to 70 % for 10 min, kept at equilibrium for 2 min, and then switched to 30 % A after 12.10 min. The flow rate was 0.3 ml/min. Chromatograms were analyzed at an integrated range between 200 nm and 500 nm. Mass spectra data were obtained in the positive ion mode by full scanning from 50 to 1, 200 m/z. A Masslynx software workstation was used for UPLC-MS instrument control, data acquisition and data processing (Liu et al. 2010).

Phytotoxicity assay

In order to assess the phytotoxicity of dyes and their degradation products, the length of plumule and radical of *Triticum aestivum* were measured (Shedbalkar et al. 2008). The measured *T. aestivum* seeds were cultured by 10 ml sterilized water (control) or a solution of degradation products. The degradation products were obtained from the ethyl acetate extraction of metabolites of dyes degradation and then dissolved in sterilized water.

Statistical analysis

All the experiments were independently performed in triplicate and differences among treatments were one-way analysis of variance (ANOVA) with a Tukey-kramer multiple comparisons test.

Results

Screening and identification

Twenty bacterial samples were isolated from sawdust to test for their potential ability to degrade aniline blue. Of these isolated strains, one strain possessed an excellent ability to decolorize crystal violet and methyl violet. This strain was identified by 16S rDNA sequencing and compared with sequences available at GenBank. The isolated strain showed a high similarity with species in the *Rhodococcus* genus and the phylogenetic tree is shown in Fig. 1. The isolated strain was deposited at the China Center for Type Culture Collection (CCTCC M 2012064), and the 16S rDNA sequence of this strain was deposited at GenBank (Accession No. JQ900306).

Effect of various culture conditions on dye decolorization

Effect of pH

The effect of pH on dye decolorization was investigated over a pH range from 4.0 to 10.0. The results showed that the decolorization of crystal violet and methyl violet was similar and the maximum decolorization was achieved between pH 5.0 - 9.0 and pH 6.0 - 9.0, respectively (Fig. 2a). The decolorization of crystal violet and methyl violet by *R. qingshengii* JB301 remained steady at a pH ranging from 6.0 to 9.0 and was inhibited at pH 4.0 and 10.0.

Effect of temperature

The effect of temperature on decolorization of the two dyes was determined at temperatures ranging from 20 to 50 °C. Figure 2b demonstrates that temperature greatly influenced

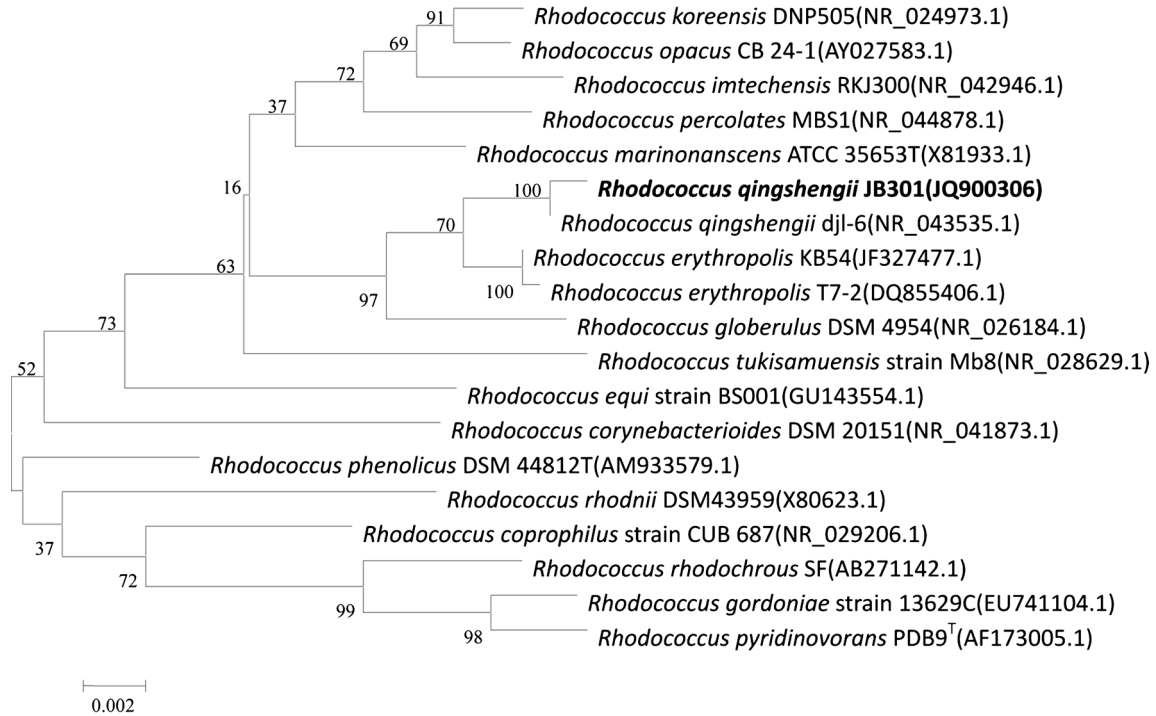


Fig. 1 Bootstrap N-J tree of *Rhodococcus qingshengii* JB301 and the relationship of strain JB301 to other members of *Rhodococcus* genus based on similarity of a 16S rRNA fragment. The scale bar indicates 0.5 % sequence dissimilarity

the decolorization of methyl violet, while it had little effect on crystal violet decolorization. The maximum decolorization of crystal violet and methyl violet were both achieved between 28 and 37 °C.

Effect of inoculation ratio

The decolorization of crystal violet remained at approximately 85 % and was slightly enhanced by increasing the inoculation ratio of *R. qingshengii* JB301 (Fig. 3a). However, the decolorization of methyl violet was greatly enhanced when the inoculation size (volume per volume) increased to 2 %.

Effect of static and shaking conditions

The 90 % decolorization of crystal violet and methyl violet at static conditions was observed after 24 h and 18 h, respectively. Compared with the static condition, the time of 90 %

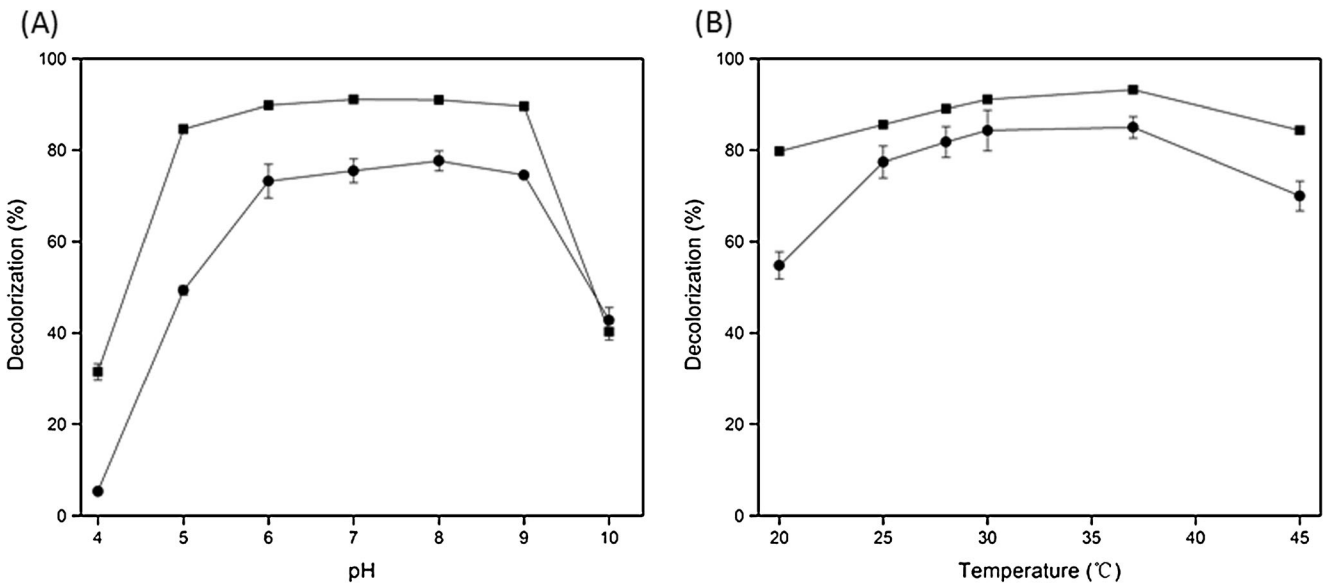


Fig. 2 Decolorization of crystal violet (■) and methyl violet (●) under different pH (**A**) and temperatures (**B**) by *Rhodococcus qingshengii* JB301

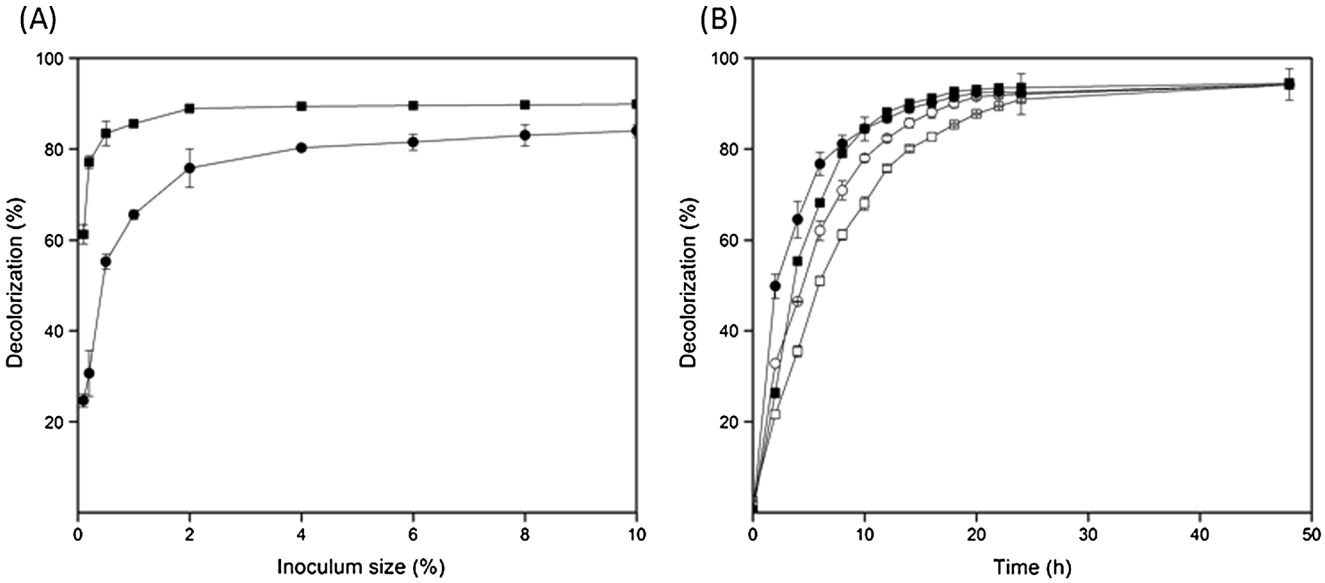


Fig. 3 Effect of inoculum size and culture conditions on dye decolorization. **A** Decolorization of crystal violet (■) and methyl violet (●) using various inoculum sizes. **B** Decolorization of crystal violet (*square*) and methyl violet (*circle*) under shaking (*filled symbols*) and static (*empty symbols*) conditions

decolorization of crystal violet and methyl violet were reduced (Fig. 3b).

Effect of dye addition time and concentration on decolorization

The effects of dye addition time and dye concentration on crystal violet and methyl violet decolorization were similar (Fig. 4). High decolorization efficiency was obtained when both dyes were added after 24 h, and the decolorization time was decreased. Figure 5 shows that the decolorization of crystal violet and methyl violet under all tested concentrations remained constant after 24 h. When the concentration of dyes exceeded 50 mg/l, the decolorization decreased sharply.

Effect of different dyes on cell growth

The effect of the added dyes on cell growth of *R. qingshengii* JB301 was investigated, and the results indicated that cell growth was inhibited by 15 mg/l crystal violet and methyl violet, compared to the control without dyes (Fig. 6).

Decolorization after repeated additions of dyes

Consecutive dye decolorization cycles were studied by repeatedly adding crystal violet (5 mg/l) and methyl violet (5 mg/l) separately. Figure 7 shows that 79.67 % decolorization of crystal violet and 85.72 % decolorization of methyl violet were achieved by *R. qingshengii* JB301 within 8 h.

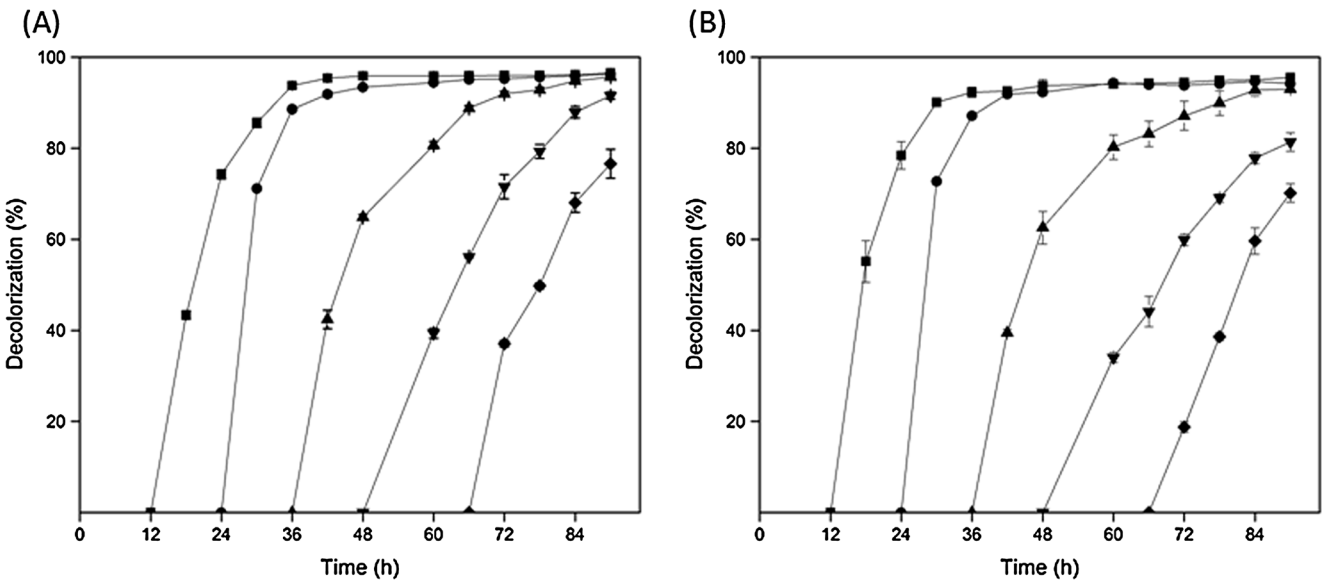


Fig. 4 Decolorization of crystal violet (**A**) and methyl violet (**B**) after different addition times: 12 h (■), 24 h (●), 36 h (▲), 48 h (▼), 60 h (◆)

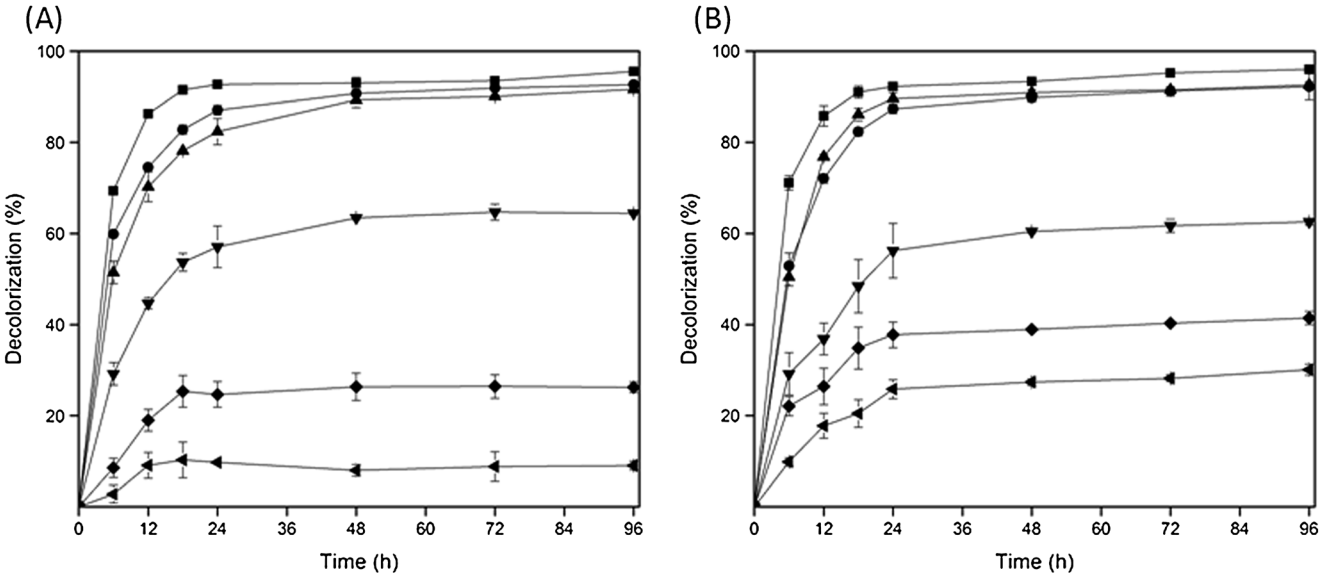


Fig. 5 Effect of different concentrations of crystal violet (**A**) and methyl violet (**B**) on decolorization. 15 mg/l (■), 30 mg/l (●), 50 mg/l (▲), 100 mg/l (▼), 150 mg/l (◆), 200 mg/l (◀)

Although the decolorization rate of both dyes was slow after five circles, a high decolorization efficiency was maintained by *R. qingshengii* JB301.

Degradation products analysis

The intermediate products of crystal violet and methyl violet during the decolorization were analyzed by LC-MS. The crystal violet intermediates were identified as leucocrystal violet with a molecular weight of 373, leucomethyl violet with a molecular weight of 359, N,N-dimethyl-N′, N′-dimethylpararosaniline (DDPR) or N,N-dimethyl-N′-methyl-N″-methylpararosaniline with a molecular weight of 345, Michler's ketone with a molecular weight of 268, [N,N-dimethylaminophenyl] [N-methylaminophenyl] benzophenone with a molecular weight of 254, [N,N-dimethylaminophenyl] [aminophenyl] benzophenone or [N-methylaminophenyl] [N-methylaminophenyl] benzophenone with a molecular weight of 240 (Table 1). The intermediates of methyl violet were similar to those of crystal violet except leucocrystal violet.

Based on these results, a degradation pathway of crystal violet and methyl violet was proposed (Fig. 8). Crystal violet was firstly demethylated to leucomethyl violet, and then formed N,N-dimethyl-N′, N′-dimethylpararosaniline or N,N-dimethyl-N′-methyl-, N″-methylpararosaniline by removal of one methyl group. These two components were split into Michler's ketone and [N,N-dimethylaminophenyl] [aminophenyl] benzophenone, or [N,N-dimethylaminophenyl] [N-methylaminophenyl] benzophenone and [N-methylaminophenyl] [N-methylaminophenyl] benzophenone, respectively.

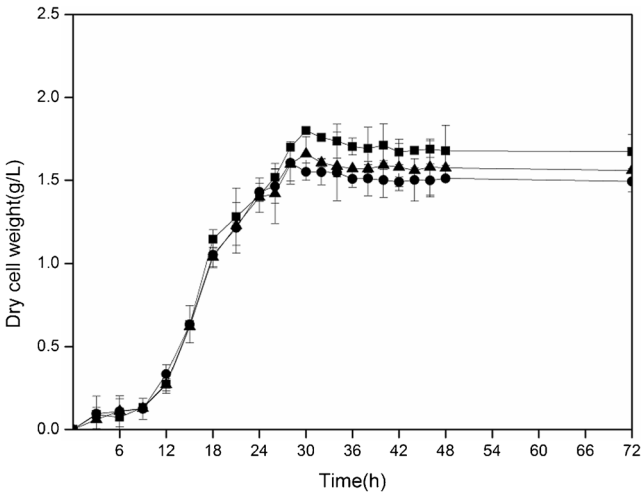


Fig. 6 Effect of culture conditions on cell growth. (●) crystal violet, (▲) methyl violet and (■) control without dyes

Analysis of enzyme activities

The activities of LiP, laccase, tyrosinase and NADH-DCIP reductase both in cell extract and supernatant were monitored before and after decolorization. Table 2 shows that only LiP and NADH-DCIP reductase were detected in the cell extract. No laccase or tyrosinase activity was detected in either cell extract or supernatant. Compared to control samples, LiP activity was greatly enhanced after addition of crystal violet (514 %) and methyl violet (698 %) after 12 h. However, reduced activity of NADH-DCIP reductase was observed during the decolorization of both dyes.

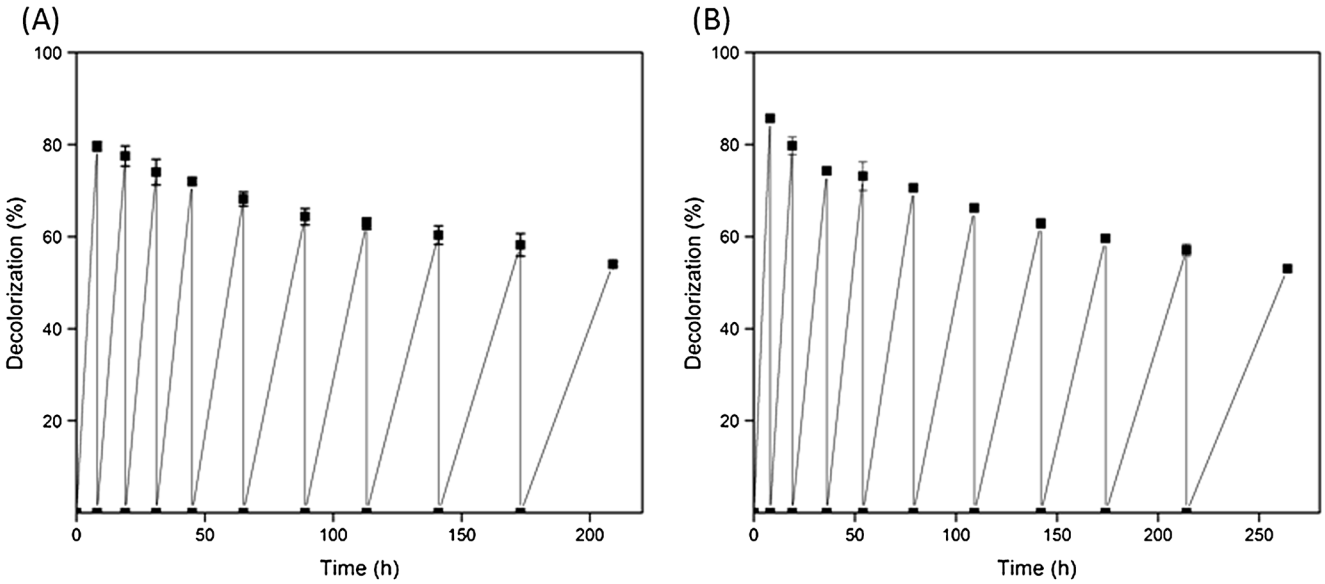


Fig. 7 Repeated use of *Rhodococcus qingshengii* JB301 in nutrient broth for decolorization of crystal violet (**A**) and methyl violet (**B**)

Phytotoxicity study

Table 3 shows that the germination of *T. aestivum* seeds was greatly inhibited by crystal violet and methyl violet when compared to metabolites obtained after decolorization and control (sterilized water). However, the plumule and radical of *T. aestivum* seeds cultured with degradation products returned to normal growth and their lengths were slightly decreased compared with the control. Thus, the toxicity of dyes could be removed by degradation through *R. qingshengii* JB301.

Discussion

A wide range of microorganisms have been reported to possess the ability to decolorize dyes. Due to their ability to produce thermostable enzymes, some fungi have been regarded as suitable candidates for decolorization (Murugesan et al. 2007). However, unlike lengthy fungal cultures, the stationary phase of bacteria can be achieved in a relatively short time under simple fermentation procedures (Maki et al. 2009). In this study, a strain highly capable of triphenylmethane dye decolorization was isolated from sawdust and was identified as *R. qingshengii* JB301. This is the first report discussing the mechanism of triphenylmethane dyes decolorization by *R. qingshengii* JB301.

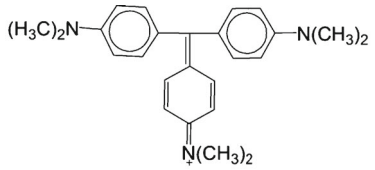
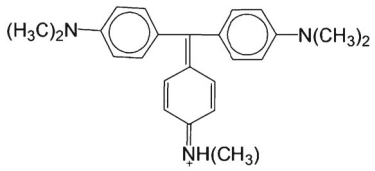
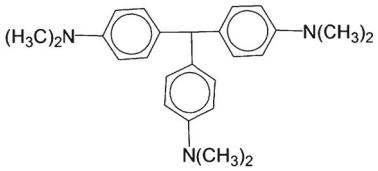
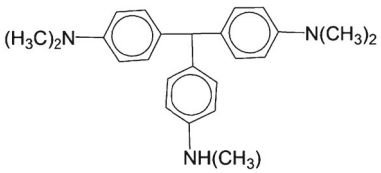
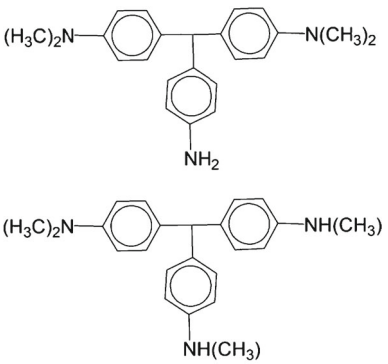
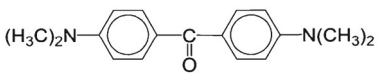
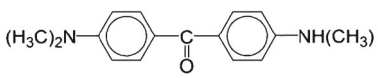
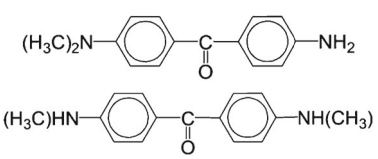
In previous studies, some members of the genus *Rhodococcus* exhibited a wide range of catabolic activities in the degradation of hazardous materials including 2,4-dinitrophenol, 2,4,6-trichlorophenol, pyridine, etc. (Briglia et al. 1996; Yoon et al. 2000a, b). Xu et al. (2007) reported that a strain possessed the ability to degrade carbendazim was identified as *R. qingshengii* sp. nov. In this work, the isolated strain showed a high homology to *R. qingshengii* sp. nov. and exhibited the capacity to degrade the methyl violet and crystal violet.

Many reports have shown that the optimal pH changed greatly for decolorization of various dyes by different microorganisms. In some bacteria, the maximum rate of decolorization was observed at the range of pH 5.0~7.0 (Zheng et al. 2009; Chen et al. 2011). In this study, the decolorization of crystal violet and methyl violet remained constant over an extensive range of pH (6.0 to 9.0), which would be favorable for bioprocessing dye-containing wastewater and useful for industrial applications (Chen et al. 2003).

In some studies, the decolorization ability of microorganisms, especially fungi, has been attributed to the secretion of efficient enzymes degrading a wide range of pollutants (Hilden et al. 2009; Maalej-Kammoun et al. 2009; Yang et al. 2011a, b). Therefore, temperature has a great influence on the decolorization ability of microorganisms by stimulating or deactivating the enzymes responsible for decolorization (Dantan-Gonzalez et al. 2008; Ayed et al. 2009). Decolorization in this study was slightly affected by temperatures ranging from 20 to 45 °C. This result indicated that the bioprocess of wastewater could be achieved at normal temperatures and, thus, decreased the costs for heating.

In this work, the biomass of *R. qingshengii* JB301 was monitored after different initial inoculum sizes, and the results indicated that the decolorization of crystal violet and methyl violet was not strongly enhanced by increasing the inoculum size above 2 %. Radha et al. (2005) reported that the removal of methyl violet increased significantly after an increase in the initial inoculum size, and a similar result was also obtained by Chen et al. (2011). However, it has been shown that cell

Table 1 Retention times and accurate mass measurements obtained from LC-MS spectra of the crystal violet and methyl violet biodegradation products by *Rhodococcus qingshengii* JB301

Intermediate products	Molecular structure	Retention time (min)	m/z(Relative intensity of predominant ions in fragmentation pattern; %)
crystal violet		7.41	372(100)
methyl violet		6.23	358(100);275(19);274(22)
Leucocrystal violet		5.01	374(100)
Leucomethyl violet		10.15	360(100); 359(7)
N,N-dimethyl-N',N'-dimethyl-pararosaniline(DDPR) or N,N-dimethyl-N'-methyl-N''-Methylpararosaniline		9.13	346(100);345(21);285(6)
Michler's Ketone		6.99	269(100);148(15)
[N,N-dimethyl-aminophenyl][N-methylaminophenyl]benzophenone		5.3	255(100);148(4); 134(7)
[N,N-dimethyl-aminophenyl] [aminophenyl] benzophenone or [N-methyl-aminophenyl][N-methyl-aminophenyl] benzophenone		3.71	241(100);239(20);215(4)

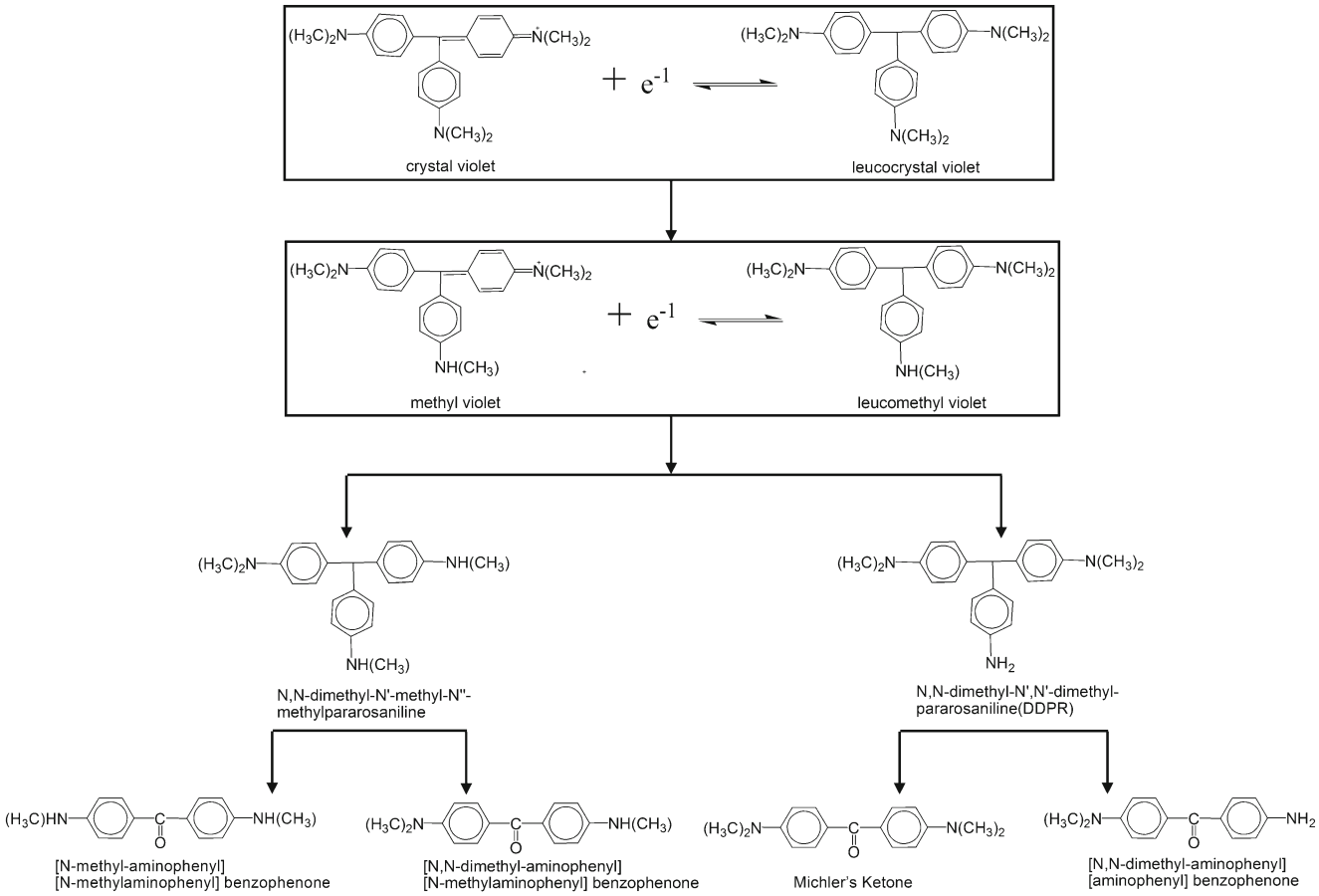


Fig. 8 Degradation pathway of crystal violet and methyl violet by *Rhodococcus qingshengii* JB301

concentrations of *Kurthia* sp. had different effects on the decolorization of effluents from textile production, and the phenomenon has been attributed to a different group of dyes (Sani and Banerjee 1999).

It has been reported that the decolorization under shaking conditions was partially or completely suppressed in comparison to static conditions (Kalme et al. 2007; Saratale et al. 2009). In a study by An et al. (2002), the removal of crystal violet by *Citrobacter* sp. under static conditions was comparatively slower than under shaking conditions, but eventually achieved the same decolorization at the end of culturing. In contrast to previous studies, our present work demonstrated that shaking conditions were beneficial to decolorization of both dyes.

The mechanisms of crystal violet and methyl violet decolorization differ for various microorganisms. Ren et al. (2006) reported that an isolated bacterium was capable of using crystal violet both as a carbon source and an energy source, and that the cell mass was greatly increased by adding crystal violet. However, the cell growth of *R. qingshengii* JB301 was

Table 2 Enzyme activities of *Rhodococcus qingshengii* JB301 during decolorization

Enzyme	The extract			Supernatant		
	Control (0 h)	Crystal violet (12 h)	Methyl violet (12 h)	Control(0 h)	Crystal violet (12 h)	Methyl violet (12 h)
Lignin peroxidase[a]	0.54±0.12	3.32±0.07*	4.31±0.10*	0.12±0.03	0.03±0.007*	0.02±0.005*
Laccase[a]	-	-	-	-	-	-
Tyrosinase[a]	-	-	-	-	-	-
NADH-DCIP reductase[b]	36.64±0.23	18.19±0.15**	21.86±0.06**	23.41±0.13	13.54±0.22*	11.17±0.38**

Data in the table are the mean of three experiments ± SEM. There was a significant difference from the control (seeds germinated in distilled water) at * $P<0.05$, ** $P<0.001$ by one-way ANOVA with Turkey comparison test

[a] Units min^{-1} L^{-1}

[b] μg of DCIP reduced min^{-1} mg $protein^{-1}$

Table 3 Phytotoxicity comparison of dyes and their extracted metabolites

Parameters studied	Water	Crystal violet (2000 ppm)	Extracted metabolite (2000 ppm)	Methyl violet (2000 ppm)	Extracted metabolite (2000 ppm)
Germination (%)	93.33	48.33	80.10	53.33	85.67
Plumule (cm)	14.52±1.13	2.95±0.10**	10.93±0.82*	5.37±0.34**	14.32±0.99***
Radical (cm)	7.16±0.56	0±0.05***	4.27±0.26**	0±0.09***	6.56±0.37**

Data in the table are the mean of three experiments ± SEM. There was a significant difference from the control (seeds germinated in water) at * $P<0.05$, ** $P<0.001$ by one-way ANOVA with Turkey comparison test

inhibited at 15 mg/l of crystal violet and 15 mg/l methyl violet. Repeated use of microorganisms for dye decolorization is important for commercial application and has been reported by several studies (Kalyani et al. 2008; Parshetti et al. 2011). The decolorization rate after the second addition of a microorganism decreased in subsequent additions and the decrease was attributed to nutrient depletion (Moosvi et al. 2005; Jadhav et al. 2008). However, *R. qingshengii* JB301 exhibited a great ability for crystal violet and methyl violet decolorization after ten rounds, which highlights its applicability in wastewater treatment processes.

As revealed by the LC-MS analysis, the same intermediates were found for both dyes. This indicated that crystal violet and methyl violet had a similar degradation pathway, which was in contrast to previous studies (Parshetti et al. 2006, 2009; Ren et al. 2006) and may be attributed to the similar chemical structure of crystal violet and methyl violet. It has been reported that crystal violet and methyl violet were reduced to their leucoderivatives during decolorization (Chen et al. 2008, 2010). Both leucocrystal violet and leucomethyl violet were detected in our analysis, indicating that these leucoderivatives were important intermediates for further dye degradation steps. Chen et al. (2010) reported that two dyes were directly split into Michler's ketone and N-dimethylamonophenol (N-methylamonophenol) by ring cleavage. However, demethylation has been found to occur before ring cleavage in methyl violet decolorization by *Aspergillus* sp. (Kumar et al. 2011). In our proposed degradation pathway, leucomethyl violet was demethylated and then degraded by ring cleavage. Crystal violet is composed of three N-dimethylaminophenol rings and the ring cleavage resulted in the same compound. Michler's ketone is the major degradation product in many crystal violet decolorizations (Yatome et al. 1991, 1993; Chen et al. 2008). In our study, four intermediates, including Michler's ketone, were detected and proposed to be produced by degradation of DDPR and N,N-dimethyl-N′-methyl-N″-methylpararosaniline. [N,N-dimethyl-aminophenyl] [N-methylaminophenyl] benzophenone has been detected in crystal violet decolorization by *Shewanella* sp., whereas [N,N-dimethyl-aminophenyl] [aminophenyl] benzophenone and [N-methyl-aminophenyl] [N-methylaminophenyl] benzophenone with a molecular weight of 240 has not been reported yet. Additionally, Chen et al. (2010) reported that small amounts of N-methylaminobenzaldehyde and N-methylaminophenol were detected during decolorization by *Shewanella decolorationis*. However, these two compounds were not detected in this study, which may be attributed to the rapid degradation.

The degradation of dyes in microbial systems is the result of enzymatic transformation (Raghukumar et al. 1996). Therefore, the enhancement of enzyme activity is beneficial to decolorizing dye effluents. Saratale et al. (2009) reported that an increased activity of NADH-DCIP reductase and azoreductase was observed during Navy blue HER decolorization. However, in this study NADH-DCIP reductase was repressed by crystal violet and methyl violet, which was similar to previously observed cotton blue decolorization by *Penicillium ochrochloron* (Shedbalkar et al. 2008). LiP is a member of Lignin-modifying enzymes (LMEs), which also contain laccase (EC 1.10.3.2), manganese peroxidase (MnP, EC 1.11.1.13) and versatile peroxidase (VP, EC 1.11.1.16) (Martínez 2002; Hammel and Cullen 2008). It has been reported that LiP possesses the ability to degrade the dyes, include Direct Blue-6 and azo dyes (Selvam et al. 2003; Kalme et al. 2007). Therefore, the increased decolorization of crystal violet and methyl violet may be caused by the enhancement of Lip activity during the fermentation. Furthermore, no enzyme activity was detected in the supernatant, indicating that the degradation of dyes was an intracellular process.

Conclusions

A newly isolated bacterium *R. qingshengii* JB301 possessed the ability to decolorize and degrade crystal violet and methyl violet. A potential degradation pathway for these two dyes was suggested to demonstrate their decolorization mechanism. [N,N-dimethylaminophenyl] [aminophenyl] benzophenone or [N-methylaminophenyl] [N-methylaminophenyl] benzophenone were detected during the decolorization, which differed from previous reports. LiP and NADH-DCIP reductase were detected in the cell extract and LiP activity was enhanced by addition of crystal violet and methyl violet. This

might be the main reason for the high decolorization efficiency of *R. qingshengii* JB301.

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