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Degradation of malachite green dye by *Tenacibaculum* sp. HMG1 isolated from Pacific deep-sea sediments

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Abstract

A deep-sea bacterium from the Pacific Ocean identified as *Tenacibaculum* sp. HMG1 was found to have strong malachite green (MG) degradation activity. The MG tolerance and decolorizing activities of strain HMG1 were confirmed by bacterial growth and high-performance liquid chromatography (HPLC) analyses. Strain HMG1 was capable of removing 98.8% of the MG in cultures within 12 h and was able to grow vigorously at 20 mg/L MG. A peroxidase gene detected in the genome of strain HMG1 was found to be involved in the MG biodegradation process. The corresponding recombinant peroxidase (rPOD) demonstrated high degradative activity at 1 000 mg/L MG. Based on the common candidate intermediates, strain HMG1 was inferred to have one primary MG degradation pathway containing rPOD. In addition, five other candidate intermediates of the rPOD-MG degradative process were detected. The optimal conditions for MG degradation were determined and showed that strain HMG1 and the rPOD enzyme could maintain high bioactivity at a low temperature (20°C), variable pH values (6.0–9.0), higher salinities (100 mmol/L) and other factors, such as multiple metal ions, H_2O_2 and EDTA. MG-tolerant strain *Tenacibaculum* sp. HMG1 and its peroxidase have prospective applications as environmental amendments for MG degradation during coastal remediation.

Key words: deep-sea sediment, *Tenacibaculum mesophilum* HMG1, peroxidase, malachite green degradation characteristics

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1 Introduction

Marine environments are one of the most diverse ecosystems due to their constantly variable environmental conditions. Consequently, marine bacteria develop the ability to adapt to extreme environmental conditions and become more capable of rapid adjustment in response to environmental changes and deterioration (Dash et al., 2013). Therefore, marine bacteria have the potential to be utilized for the bioremediation of recalcitrant chemicals via precipitation, volatilization, energy-dependent efflux systems, intracellular sequestration by proteins, enzymatic degradation, biofilm formation, etc. (Sakalle and Rajkumar, 2009; Vu et al., 2009; Von et al., 2002).

Dyes typically have complex aromatic c molecular structures that make them more stable and more difficult to biodegrade. Global industrialization has resulted in the widespread contamination of marine environments through the constant addition of organic and inorganic wastes. Malachite Green (MG) is a synthetic triphenylmethane dye that is extensively used in the textile industry. In addition, MG was widely used in aquaculture as a parasiticide and fungicide. Although the use of MG has been banned in several countries due to its health risks, which include effects on the immune and reproductive systems as well as genotoxic and carcinogenic properties, it is still being used in many areas worldwide due to its low cost, ready availability and high efficacy (Cha et al., 2001; Srivastava et al., 2004; Zhai et al., 2007). Effluents with MG also reduce light penetration and substantially increase the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which reduce the biodiversity and capabilities of aquatic ecosystems (Novič et al., 1988; Garg et al., 2004; Darajeh et al., 2014). Moreover, the MG that remains accumulates in animal bodies and is transferred to humans through the food chain. As a consequence, various methods to remove this dye from aqueous solutions have been studied, and they can be divided into physical adsorption, chemical destruction and biological degradation methods.

Utilizing the metabolic potential of microorganisms to degrade or transform organic contaminants into less harmful substances that can be integrated into natural biogeochemical cycles is the important goal of bioremediation technology. In recent years, biodegradation methods have become appealing approaches for decomposing this recalcitrant compound. Microorganisms with the ability to degrade MG have been reported, such as *Micrococcus* (Du et al., 2013), *Saccharomyces cerevisiae* (Jadhav and Govindwar, 2006), *Pseudomonas* (Du et al., 2011; Tao et al., 2017), and *Kocuria rosea* (Parshetti et al., 2006). Desmethyl malachite green, leucomalachite green, desmethyl leucomalachite green and N, N-dimethylaniline are the common metabolic intermediates of MG biodegradation (Du et al., 2011;

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Du et al., 2013; Wang et al., 2012). However, most reported microbial strains were isolated from soil and freshwater. Therefore, the property of these strains that low physiological activities in marine environments could limit capacities for the treatment of coastal MG pollution, compared with marine-derived microorganisms. Alternatively, there have been sporadic reports of the degradation of dyes by marine-derived enzymes or fungi. Torres et al. (2011) found that some marine fungal strains were capable of discoloring and adsorbing dyes after 3–4 weeks of incubation at room temperature, and Raghukumar et al. (2008) treated colored effluents with lignin-degrading enzymes derived from marine fungi.

In the present study, a marine bacterium that showed relatively high MG degradation activity was isolated from deep-sea sediments in the Pacific Ocean. The MG degradation-related enzyme peroxidase was detected, and recombinant peroxidase (rPOD) expressed in *E. coli* BL21 (DE3) cells was verified to rapidly degrade high concentrations of MG. In addition, the pathway and characteristics of MG degradation were analyzed to further understand the MG degradation process and the harmfulness of its products. These experimental data revealed that the strain and its enzyme have bioremediation potential for the removal of MG from aqueous solutions and marine environments.

2 Materials and methods

2.1 *Samples*

Samples were collected from Pacific deep-sea sediment at Site 22IX-S026-CC1109-TVMC14 (8°53′08″N, 142°59′51″E; water depth, 5 493 m) using a gravity corer. Approximately 3 cm of sediment was collected from the bottom of the gravity corer. Under sterile conditions, the surface of the sample was immediately discarded, and the remainder was stored at 4°C. The surface of the sample was again discarded after it was transported to the lab, and only the central section of the sample was subjected to further analysis.

2.2 *Isolation and identification of the MG-degrading bacterial strain HMG1*

The isolation of strain HMG1 for MG decolorization was conducted using the following protocol. First, 50 mL of liquid 2216E medium with 5 mg/L MG (Sangon Biotech Co., China) were mixed with 5 g of deep-sea sediment, and the mixture was incubated at room temperature until its green color faded. Next, 10 μL of the culture supernatant was inoculated into another 50 mL of fresh liquid 2216E medium with 10 mg/L MG, further enriching the domesticated MG-degrading strains. Finally, 10 μL of the supernatant from the second culture was diluted (10^{-4}), plated on 2216E agar and incubated overnight at room temperature. One strain that grew on the MG screening plates was observed to have greater zones of clearing than the other strains, suggesting that it had the strongest MG degradation activity. This decolorizing strain was named HMG1, and a single colony was selected, cultivated and identified by 16S rRNA gene sequence analysis (Weisburg et al., 1991).

2.3 *Decolorization experiments with strain HMG1*

The decolorization efficiency of strain HMG1 was measured using high-performance liquid chromatography (HPLC). Strain HMG1 bacterial cells were cultured in 2216E liquid medium at 30°C with shaking, and then an additional 20 mg/L of MG was added under static conditions. An aliquot of the broth was collected at different time intervals, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h and 28 h, and extracted with dichloromethane (chromatographic grade, Sigma-Aldrich Co., USA) three times. The extracts were evaporated to dryness at 30°C and further dissolved in 1 mL of acetonitrile (chromatographic grade, Sigma-Aldrich Co. LLC, USA). The HPLC system (LC-20a Series, Shimadzu, Japan) was equipped with a C18 analytical column (4.6 mm×250 mm, 5 μm, LC Sciences, Japan), and measurements were performed using a mobile phase containing acetonitrile (chromatographic grade, Sigma-Aldrich Co. LLC, USA) and 0.125 mol/L ammonium (pH 4.5, Sinopharm Chemical Reagent Co., China) (80:20 (v/v)) at a flow rate of 1.0 mL/min. The detection wavelength was 622 nm, and the injection volume was 20 μL (Ren et al., 2007). The decolorization efficiency (E_d, %) was calculated by the following formula: $E_d = (A–B)/A \times 100$, where A is the initial peak area of MG, and B is the final peak area of MG. All experiments were performed in triplicate.

Cultures of HMG1 with different concentrations of MG (0 mg/L, 20 mg/L, 100 mg/L or 1 000 mg/L) were incubated for different durations (4 h, 8 h, 12 h, 16 h, 20 h, 24 h and 28 h), and a 10-μL aliquot from each culture was diluted 10^8-fold and spread on 2216E agar plates. The number of colonies after overnight incubation at 30°C was counted to determine the influence of MG on the viability of strain HMG1.

2.4 *Identification of the peroxidase gene in the HMG1 genome using draft genome sequencing*

The genome sequence of HMG1 was determined using an Illumina HiSeq2000 platform at Shanghai Majorbio Bio-Pharm Technology Co. (China). All of the reads were assembled with SOAPdenovo, and the open reading frames (ORFs) were predicted with Glimmer 3.0. The rRNA and tRNA genes were evaluated using the RNAmmer and tRNAscan-SE servers, respectively. The scaffolds were searched against the SWISSPROT database to identify enzymes potentially involved in MG biodegradation. A phylogenetic analysis was performed by building a Neighbor-Joining tree with the MEGA5.10 program using target ORFs and similar proteins from 15 other microbes.

2.5 *Enzyme expression and purification*

A peroxidase gene detected in the HMG1 genome was further cloned using primers designed according to the ORF sequence. The forward and reverse primers were 5′-AACGTCGACAAAA-AAAAATAATCTTAAGCGTAGTAACAGC-3′ and 5′-ATCGCGGC-CGCAAGACTTCAAATCAAACCTATCTAGG-3′ and contained a *Sal*I and *Not*I site, respectively. The PCR products were ligated into the pET-32a vector (Novagen, US) and transformed into *E. coli* BL21 (DE3) cells. Positive clones were grown in LB medium supplemented with ampicillin (100 μg/mL) and glucose (0.7%) at 37°C with vigorous shaking to an OD_{600} of approximately 0.5. Expression of the peroxidase was then induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to the culture at a final concentration of 1 mmol/L, followed by incubation at 18°C for 20 h. The cells were harvested and ultrasonicated, and rPOD was purified via nickel-affinity chromatography by elution with 200 mmol/L imidazole. The rPOD was used for the determination of MG degradation activity.

2.6 *Characterization of rPOD decolorizing activity*

The decolorization efficiency of MG by rPOD was determined by UV-visible analysis with a UV752N spectrophotometer (Shanghai Yok Instruments Co., China). The reaction mixture, which included 100 μL of purified rPOD, 1% H_2O_2, and MG (20 mg/L, 100 mg/L or 1 000 mg/L) in 20 mmol/L Tris-HCl (pH 8.0),

was incubated at 30°C, and the absorbance of the supernatant at 622 nm was measured at different time intervals, 0.5 h, 1 h, 2 h, 3 h and 4 h. The decolorization efficiency was calculated using the same formula as in the decolorization experiments, except that A and B are the initial and final absorbances, respectively.

The effect of temperature on the decolorizing efficiency was determined by measuring the decolorization percentage of 100 mg/L MG in 0.5 h at 20°C, 30°C, 40°C, 50°C and 60°C. Similarly, the effect of pH was determined at 30°C in acetate-NaOH (pH 3.0–6.0), phosphate-NaOH (pH 7.0–8.0), and Tris-HCl (pH 9.0–10.0) (Zhang and Zeng, 2008; Chi et al., 2014; Han et al., 2016) buffers. The effect of H_2O_2 was determined at 30°C by adding 0%, 0.5%, 1%, 2% and 3% H_2O_2 to the reaction mixture. The effect of metal ions was determined by adding $ZnSO_4$, $MgSO_4$, $MnSO_4$, $NiSO_4$, $FeSO_4$, $CuSO_4$, $Al_2(SO_4)_3$ and $CaSO_4$ at final concentrations of 1 mmol/L to the reaction mixture. The effect of potential inhibitors was determined by adding NaCl (1 mmol/L, 10 mmol/L and 100 mmol/L), EDTA (1 mmol/L, 10 mmol/L and 100 mmol/L) and SDS (0.1% and 1%) to the reaction mixture. All experiments were performed in triplicates.

2.7 *Determination of MG biodegradation intermediates by LC-MS*

To determine the MG biodegradation intermediates of strain HMG1, 20 mg/L MG was added to a cell culture and incubated at 30°C for approximately 10 h. The fermentation broth was lyophilized and extracted with methanol (chromatographic grade, Sigma-Aldrich Co., USA) in the dark. To determine the MG biodegradation intermediates of rPOD, the same operation was performed, except incubation times of 15 min and 45 min were used.

All of the samples were analyzed using LC-MS (1290 LC 6490 QQQ, Agilent Technologies, USA) with an ESI ion source. The mobile phase contained ammonium (20 mmol/L, pH 4.5) and acetonitrile. The initial proportion of acetonitrile was 10%, and it reached 100% within 50 min. The injection volume was 5 μL, and the default settings were used for the other parameters.

2.8 *Gene accession number*

The nucleotide sequence of the 16S rRNA gene and peroxidase gene from strain HMG1 have been deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers KM047889 and KM047890, respectively. Genomic sequences of HMG1 have been deposited in the GenBank database under the accession number LDOD00000000.

3 Results

3.1 *Identification of strain HMG1 and characterization of its decolorization activities*

Strain HMG1 had an irregular colony shape with a spreading edge and exhibited the strongest decolorizing activity on the screening medium. The 16S rRNA gene sequence showed 99% similarity with *Tenacibaculum* sp. MBIC1140. Examination of its decolorizing activities affirmed that strain HMG1 was capable of quickly decolorizing MG at 20 mg/L, and the decolorization rate increased nearly linearly from 0 h to 4 h (Fig. 1). At an initial concentration of 20 mg/L, after 4 h, HMG1 had degraded 94.9% of the MG. Afterwards, the MG degradation rate slowed, but incubations of HMGI with 20 mg/L MG for 8 h, 12 h, 16 h, 20 h, 24 h and 28 h resulted in 97.3%, 98.8%, 99.2%, 99.3%, 99.4% and 99.6% MG decolorization efficiency, respectively. High concentrations of MG (100 mg/L and 1 000 mg/L) substantially inhibited bacterial growth, whereas the number of colony forming units per microliter (CFU/μL) increased markedly at 20 mg/L MG. The number of CFU/μL in the control group declined after 8 h (approximately 4.73×10^9 colonies); however, the number of CFU/μL continued to increase up to 16 h at a concentration of 20 mg/L of MG (approximately 1.06×10^{10} colonies).

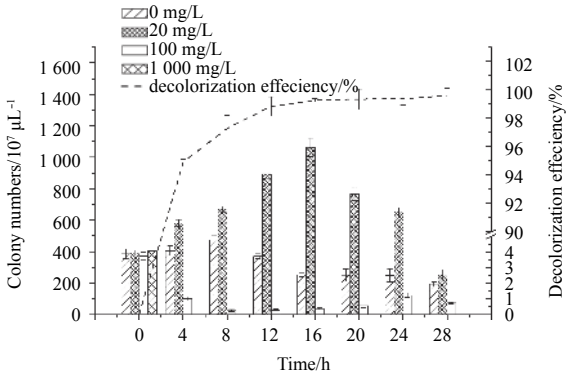

Fig. 1. The decolorization efficiency of strain HMG1, and the influence of MG on its viability.

3.2 *Sequence analysis of bacterial genome and MG-degrading enzyme of MG-degrading enzymes*

The draft genome of *Tenacibaculum* sp. HMG1 consists of 3 420 298 bases and has a G+C content of 31.55%. All of the reads were assembled into 90 contigs in 65 scaffolds. Overall, there are ten genes that encode tRNAs, one gene that encodes 5S rRNA and one gene that encodes 16S rRNA. In addition, 3 111 putative ORFs were found, giving a genes/genome percentage of 88.4%. A peroxidase gene, which, according to previous references, most likely confers MG degradation ability to HMG1 (Bhunia et al., 2001; De Souza et al., 2007), was found in the draft genome and had the highest amino acid identity (68%) to *Burkholderia thailandensis* E264. However, a phylogenetic tree based on amino acid sequences showed that the *Tenacibaculum* sp. HMG1 peroxidase, the corresponding protein of the peroxidase gene, clustered with that of *Geobacillus thermodenitrificans* NG80-2 and was distinct from *B. thailandensis* (Fig. A1).

3.3 *Purification of rPOD and characterization of its MG decolorization*

The peroxidase gene was cloned into the pET-32a vector for heterologous expression. The rPOD, with molecular weight of 105 kDa (Fig. 2a), showed much higher MG decolorizing ability than strain HMG1. More than 90% of MG was removed within 0.5 h at different concentrations of MG, and rPOD could degrade 96.07% of MG within 4 h at the rather high concentration of 1 000 mg/L (Fig. 2b). The optimum temperature of rPOD for MG decolorization was 30°C, which resulted in 98.73% decolorization efficiency. Moreover, high degradation activity was maintained from 20°C to 30°C, and the efficiency was still 98.65% at 20°C (Fig. 2c). rPOD exhibited high MG decolorizing activity in the wide pH range of 6.0–9.0, with an optimum pH of 8.0 (Fig. 2d). H_2O_2 greatly impacted the activity of rPOD, as an MG degradation efficiency of 99.02% was obtained with 0.5% H_2O_2, but only 33% efficiency was achieved without H_2O_2 (Fig. 2e). Small changes in decolorization efficiency showed that rPOD was not sensitive to the metal ions tested in this study (Fig. 2f). Similarly, none of the tested concentrations of EDTA (1 mmol/L, 10 mmol/L and 100 mmol/L) or NaCl (1 mmol/L, 10 mmol/L and 100 mmol/L) had a significant effect on the decolorization efficiency, which still reached approximately 98% in each case. In contrast, the decol-

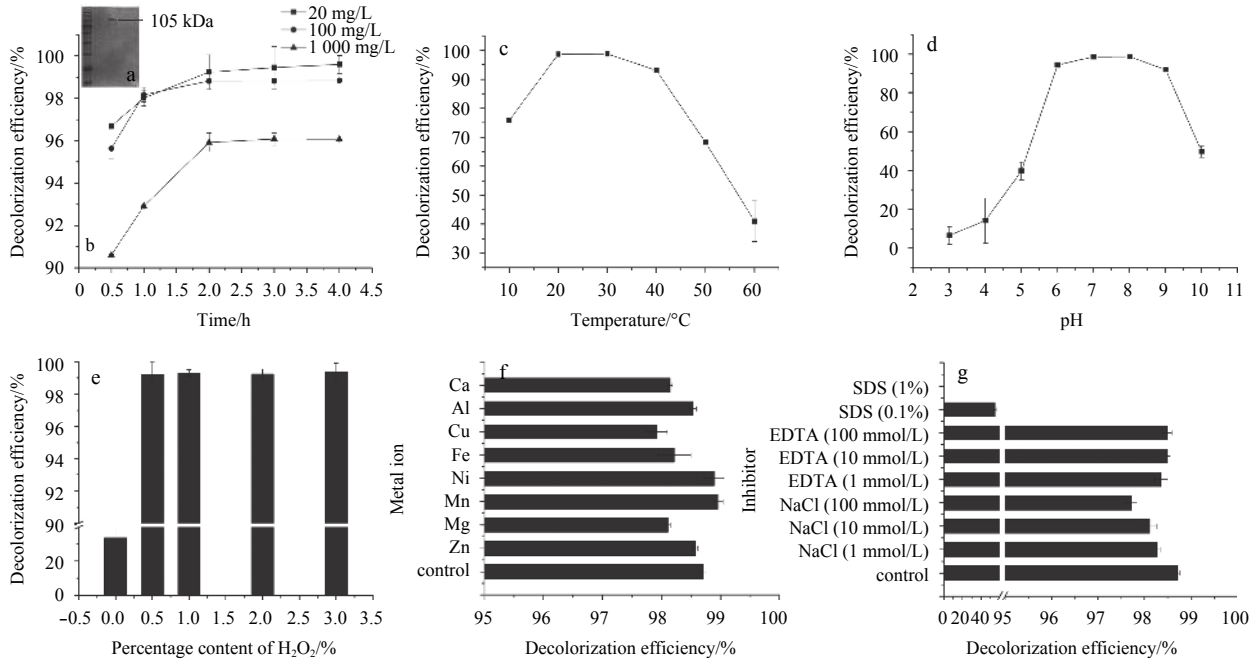

Fig. 2. The influence of assay conditions on the decolorization efficiency of rPOD.

orization efficiency of MG decreased rapidly with 0.1% SDS (54.8%) and 1% SDS (0%) (Fig. 2g).

3.4 *MG biodegradation intermediates*

Using LC-MS, three and eight intermediates were found in the MG degradation process of strain HMG1 and enzyme rPOD, respectively. The candidate products were identified according to their m/z values (Fig. A2). The three peaks detected for strain HMG1 represented desmethyl-MG (m/z 315), 4-(dimethylamino) benzophenone (m/z 226), and 4-(methylamino) benzophenone (m/z 212). In addition to the above peaks, the intermediates for rPOD also included didesmethyl-MG (m/z 302), hydroxyl-MG (m/z 346), hydroxyl (didesmethyl)-MG (m/z 318), Michler's ketone (m/z 269) and N, N-dimethylaniline (m/z 122).

3.5 *Analysis of the MG biodegradation pathway*

Two possible MG degradation pathways were deduced based on the detected intermediates (Fig. 3). In the first pathway, which could be involved in the degradation process of both strain HMG1 and enzyme rPOD, two demethylation reactions are catalyzed in the first step of degradation to produce didesmethyl-MG (m/z 302). Subsequently, peroxidase seizes an electron from the central carbon of MG, and water or hydrogen peroxide serves as a hydroxylation agent (Goszczynski S et al., 1994), transforming MG into a hydroxylated intermediate (m/z 318). Then, a nucleophilic reaction acts on the dye to generate 4-(dimethylamino) benzophenone (m/z 226), releasing a benzene ring-containing compound as the leaving group. Ultimately, another demethylation occurs, and 4-(methylamino) benzophenone (m/z 212) is generated for further degradation.

The intermediates detected from the rPOD degradation reactions indicate another pathway for MG degradation. In this pathway, hydroxylation first appears to produce hydroxyl-MG (m/z 346). Afterwards, the central carbon is attacked to generate Michler's ketone (m/z 269) and 4-(dimethylamino) benzophenone (m/z 226) due to the different leaving groups. The follow-up reaction of 4-(dimethylamino) benzophenone (m/z 226) involves the above pathway. Moreover, the carbonyl of Michler's ketone (m/z 269) would then break to generate N, N-dimethylaniline (m/z 122) for further degradation.

4 Discussion

Wastewaters discharged from different industries, such as textiles, leather tanning, paper production, aquaculture farming, food technology, and hair coloring, are usually polluted with dyes (Robinson et al., 2001). Among them, MG is most commonly used in different areas. The conventional methods for treating dyes in coastal pollution areas, including oxidation, membrane separation, activated carbon adsorption and seaweed absorption, do not show considerable efficiency or economic advantages for use in the marine environment. MG can also be transformed by zooblasts, such as *Cunninghamella elegans* and channel catfish muscle, but the degradation products, namely, leucomalachite green (LMG) and leucogentian violet (LGV) (Cha et al., 2001; Chen and Miao, 2010), are still mutagenic and carcinogenic (Littlefield et al., 1985). One promising strategy to remediate MG pollution is the application of microbes, which are environmentally friendly, for the degradation of toxic compounds due to low running costs and nontoxic mineralized end products (Forgacsa et al., 2004). In this study, *Tenacibaculum* sp. HMG1 was isolated from marine sediments and identified as a member of the Cytophaga-Flavobacterium-Bacteroides (CFB) group (Pinhassi et al., 1997). HMG1 is a potential candidate for the biological treatment of polluted extreme habitats because of its high adaptability and MG degrading efficiency in marine environments.

A redox reaction is essential for the degradation of MG, and various oxidases and reductases, including laccase, cytochrome oxidase P450, TMR, TpmD and peroxidase (Zhang et al., 2009), have been reported to be responsible for triphenylmethane dye decolorization by microorganisms. To protect organisms from. OH radicals, peroxidases can remove hydrogen peroxide by transferring electrons from the donor to H_2O_2 to produce hydron (Poulos and Kraut, 1980). Peroxidases from plants and microor-

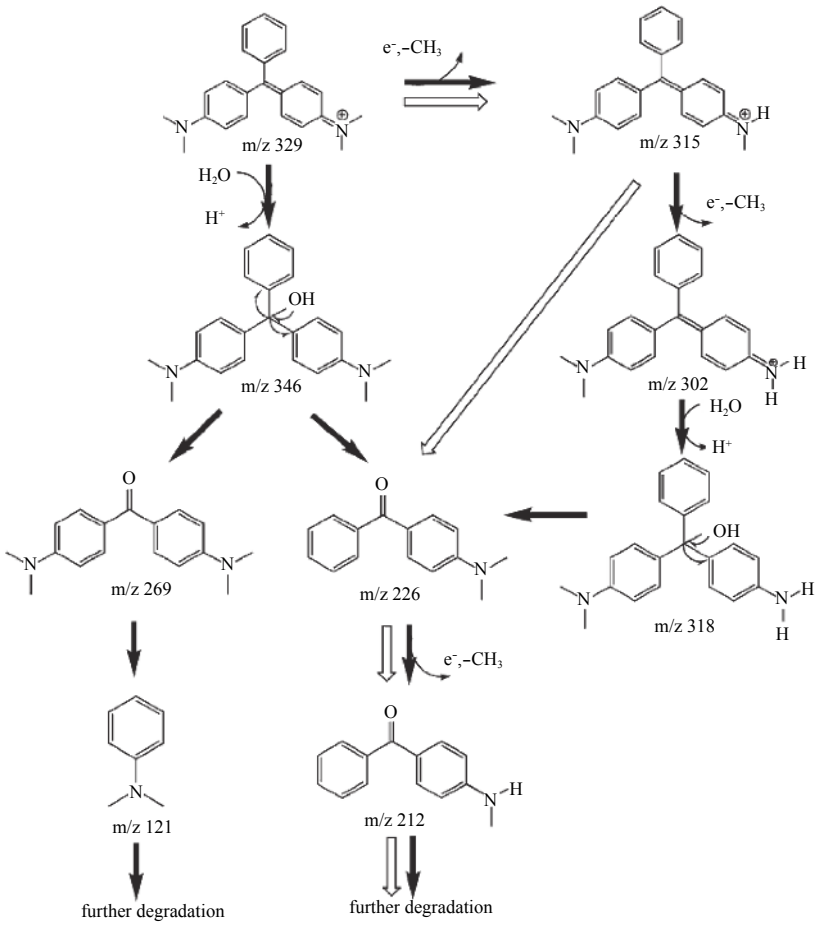

Fig. 3. The deduced MG degradation pathways determined from the intermediates of strain HMG1 and enzyme rPOD.

ganisms have been shown to have many applications in the degradation of biological macromolecules, such as industrial dyes (Bhunia et al., 2001; Kuhad et al., 2013; Ali et al., 2013). In this study, a peroxidase gene from strain HMG1 was identified by draft genome sequencing, and its MG decolorization activity under different conditions was further verified with recombinant enzyme expressed in *E. coli* BL21 (DE3) cells. The high decolorization activity and shared degradative intermediates demonstrated that this peroxidase likely participates in the biodegradation of MG in HMG1.

A possible MG degradation pathway was proposed based on the intermediates determined by LC-MS. The MG degradation pathway of strain HMG1 is consistent with previous investigations that showed that reductive splitting of the triphenylene rings begins with methyl group removal from the parent structure in *Shewanella decolorationis* NTOU1 (Chen et al., 2010). All possible mechanisms for the N-demethylation of N, N-dimethylamine by peroxidase were described in a previous report (Miva et al., 1983; Kedderis et al., 1983; Kedderis and Hollenberg, 1983). In addition, previous investigations have hypothesized that this process could influence the rates of the subsequent reactions (Du et al., 2013; Chen et al., 2010). An rPOD degradation pathway beginning with demethylation is consistent with the degradation process of strain HMG1, but another pathway initiated by hydroxylation instead of demethylation was also detected for the rPOD degradation process. Differences between the degradation pathways of the strain and enzyme implied that some of the intermediate products probably entered other basal metabolic pathways of the bacterium. These results also further demonstrated that the MG process occurs through degradation rather than absorption.

Interestingly, after adding MG at 20 mg/L, the number of CFU/μL of strain HMG1 increased sharply from 3.92×10^9 to 1.062×10^{10}, suggesting that at a certain concentration, MG could be used as a nutrient source to promote HMG1 growth. In addition, the fourfold increase in growth compared with the control group suggested the strong adaptability of strain HMG1 to an environment containing a moderate level of MG. High degradation activity (98.6%, 20 mg/L MG) at a low temperature (20°C) is also beneficial for the application of rPOD due to the typically low temperature of natural seawater (approximately 10–35°C) (Xia and Gu, 2000). rPOD was not strongly affected by environmental interference, including metal ions, EDTA (from 1 mmol/L to 100 mmol/L), NaCl (from 1 mmol/L to 100 mmol/L) and different pH values (from 6.0 to 9.0), and thus shows promise for removing MG from complex waste seawater at low temperatures, high salinities and variable pH values.

Compared with other marine fungi or freshwater bacteria, strain HMG1 and enzyme rPOD demonstrated higher MG degradation efficiency. These superior properties of strain HMG1 and enzyme rPOD in the degradation of MG indicate that they could represent good options for the remediation of MG pollution in marine environments.

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Appendix:

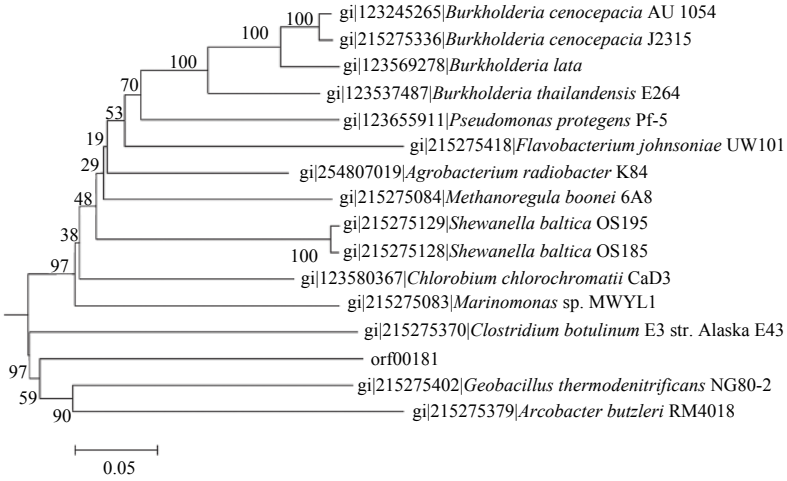

Fig. A1. Phylogenetic tree of the peroxidase from ORF 00181. Bootstrap values of 500, calculated from 1 000 bootstrap trees, are indicated at the nodes. The numbers in the brackets are the GenBank accession numbers of the referenced amino acid sequences. The scale bar represents 0.05 amino acid substitutions per position.

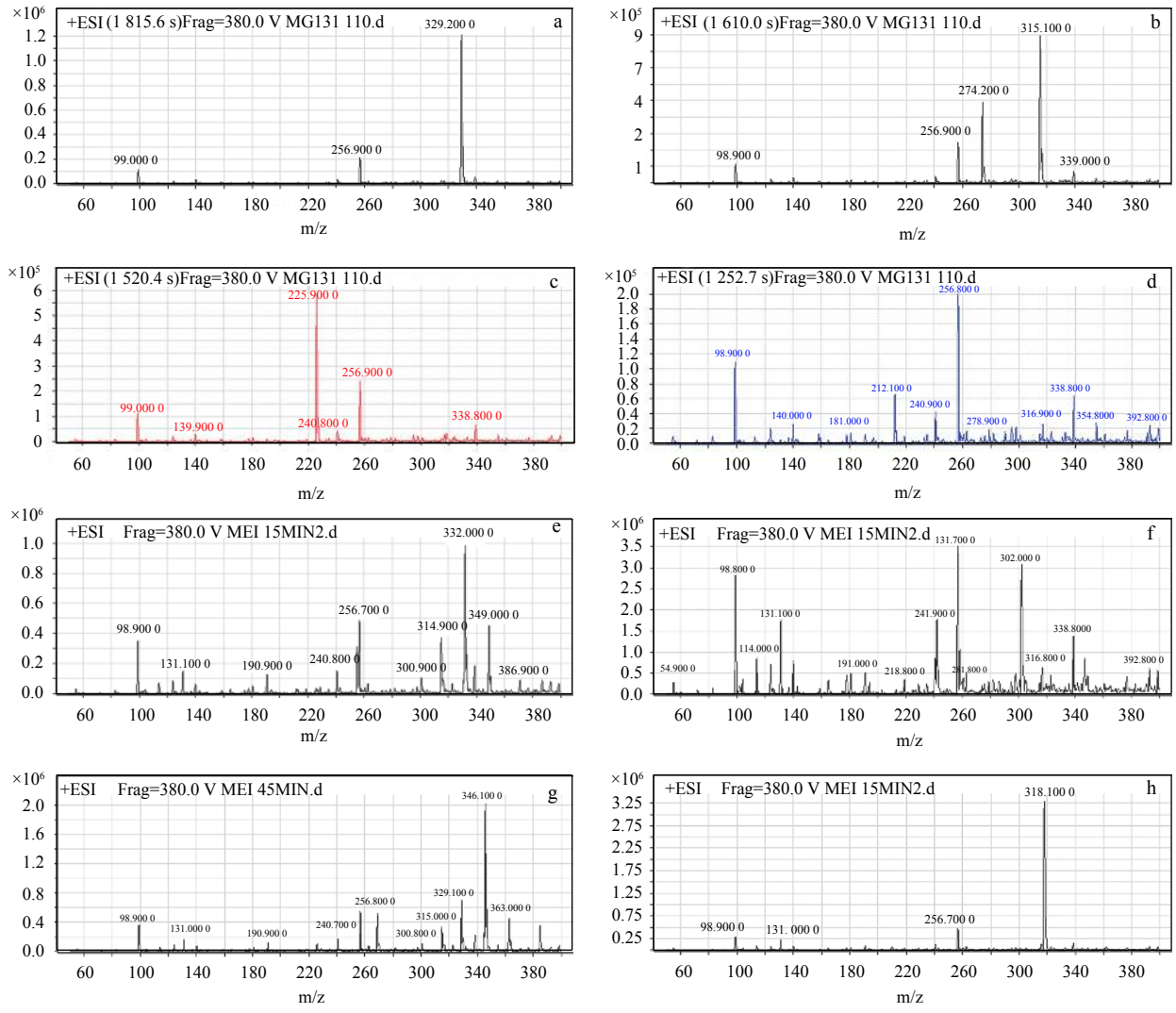

Fig. A2

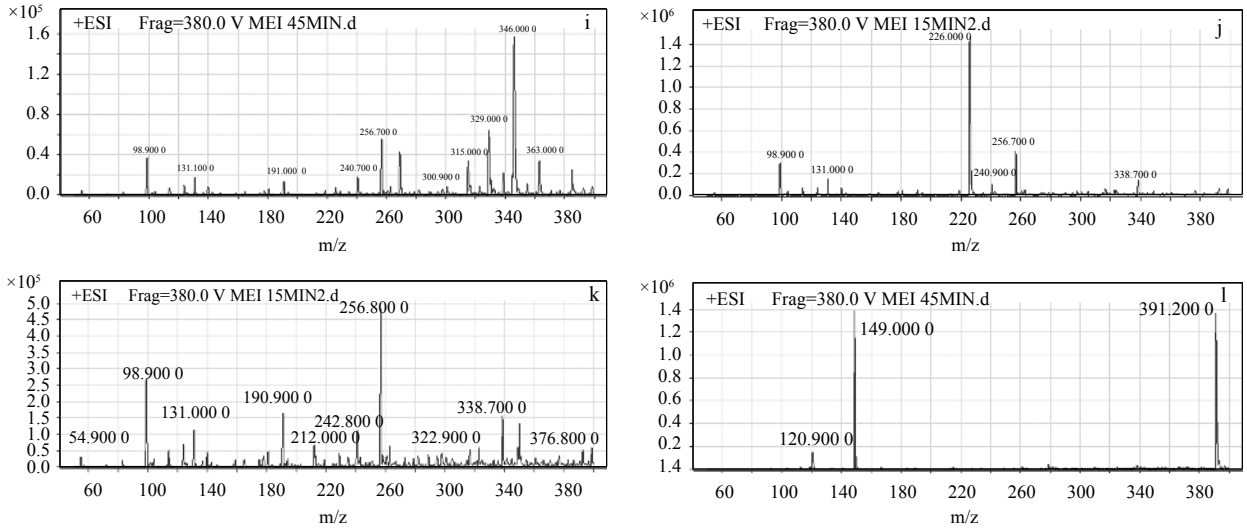

Fig. A2. LC-MS results of the probable intermediates of MG degradation. a. MG (m/z 329), b. desmethyl-MG (m/z 315), c. 4-(dimethylamino) benzophenone (m/z 226), d. 4-(methylamino) benzophenone (m/z 212), e. desmethyl-MG (m/z 315), f. didesmethyl-MG (m/z 302), g. hydroxyl-MG (m/z 346), h. hydroxyl didesmethyl-MG (m/z 318), i. Michler's ketone (m/z 269), j. 4-(dimethylamino) benzophenone (m/z 226), k. 4- (methylamino) benzophenone (m/z 212), and l. N, N-dimethylaniline (m/z 122).