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Biodegradation and detoxification of endosulfan in aqueous medium and soil by *Achromobacter xylosoxidans* strain CS5

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ABSTRACT

Achromobacter xylosoxidans CS5, capable of utilizing endosulfan as the sole carbon, sulfur and energy source, was isolated from the activated sludge. Degradation of endosulfan by strain CS5 was examined by HPLC. Analysis of culture pH, cells growth, and residual endosulfan demonstrated that CS5 could degrade more than 24.8 mg/l α-endosulfan and 10.5 mg/l β-endosulfan after 8 days in aqueous medium, with the formation of endosulfan diol and endosulfan ether as the major metabolites. Cell-free extract of strain CS5 was able to metabolize endosulfan rapidly, and the degradative enzymes were constitutively expressed. Inoculation of strain CS5 was found to promote the removal of endosulfan in soil. Our results suggested that *A. xylosoxidans* CS5 might degrade endosulfan by a non-oxidative pathway. In addition, detoxification of endosulfan was evaluated using a *Salmonella typhimurium* TA1535/pSK1002 (*umu*-test). These finding suggested that the metabolism of endosulfan by strain CS5 was accompanied by significant reduction in the toxicity.

1. Introduction

Endosulfan is a broad-spectrum cyclodiene insecticide that has been used extensively for over 30 years on a variety of crops [1]. Technical endosulfan is synthesized as a mixture of two isomers approximately 70% α-endosulfan and 30% β-endosulfan. Since endosulfan and its breakdown products are persistent in the environment with an estimated half-life of 0.7–6 years [2]. Contamination and persistence of endosulfan in aquatic and soil environments lead to accumulation in crop, tea, phytoplankton, zooplankton, fishes and vegetables [3–6]. It is extremely toxic to fish and aquatic invertebrates and affects the central nervous system, kidney, liver, blood chemistry and parathyroid gland, which has reproductive, teratogenic, and mutagenic effects [7–9]. These health and environment concerns have led to an interest in detoxification of endosulfan in the environment.

Degradation of endosulfan through biological means is receiving serious attention as compared to existing methods such as incineration and landfill. In biodegradation progress, heterotrophic microorganisms break down substrates (hazardous compound) to obtain chemical energy, hence organic pollutants can serve as carbon, energy, and nutrient sources for microbial growth [10]. Previous researchers have reported that endosulfan could be used as a sole sulfur source for microbial growth [1,11,12], or as a sole carbon source in biodegradation test [10,13–17]. In biodegradation, endosulfan can be degraded by attacking the sulfite group via either oxidation to form the toxic metabolite endosulfan sulfate or hydrolysis to form the less toxic metabolite endosulfan diol [1,11,15,17,18]. Endosulfan diol is a non-toxic metabolite to fish and other organisms. It can be further degraded to non-toxic endosulfan ether, endosulfan hydroxyether and endosulfan lactone; thus, production of endosulfan diol via hydrolysis may be an important detoxification pathway of endosulfan [9,19]. Some investigators have reported that the toxicity of endosulfan residues and the metabolites formed after degradation was evaluated by the mortality of the test organisms *Tubifex tubifex* [16], by yeast-based cell growth inhibition [14] and by the frequency of micronucleus (MN) emergence in polymorphonuclear leukocyte cells [13]. However, a simple, inexpensive and sensitive system (*umu*-test) [20] for the evaluating the genotoxicity of the residual of endosulfan and the metabolites formed during degradation is still needed.

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Many researchers have reported that endosulfan was degraded by microorganisms in aqueous medium and in soil [1,15,17,18,21–25]. However, no report on the enzymatic degradation of endosulfan by cell-free extracts of strain has been published so far. Therefore, the purpose of our work was to isolate a bacterium which was able to degrade of endosulfan in aqueous medium and soil. The enzymatic degradation by cell-free extracts and its detoxification using *umu*-test in aqueous medium and soil were also assessed.

2. Materials and methods

2.1. Media and chemicals

Technical grade endosulfan (>95% purity) was obtained from Jiangsu Anpon Pesticides Co., Ltd., China. Endosulfan diol, endosulfan ether, endosulfan sulfate and endosulfan lactone standards (99.5% purity) were purchased from the Laboratories of Dr. Ehrenstorfer, Augsburg, Germany. The enzyme substrate *O*-nitrophenyl-β-D-galactopyranoside (ONPG) was purchased from Amresco (USA). Luria–Bertani broth and TGA medium (tryptone, 10 g/l; NaCl, 5 g/l; glucose, 2 g/l; ampicillin, 20 mg/l) were used in *umu*-test [20]. The enrichment medium TYC (tryptone, 5.0 g/l; yeast extract, 5.0 g/l; KH_2PO_4, 1.0 g/l) was used and prepared in enrichment test. The minimal medium (MM) had the following composition: KH_2PO_4, 2.0 g/l; K_2HPO_4, 7.5 g/l; NH_4Cl, 1.0 g/l; NaCl, 0.5 g/l; $MgCl_2$, 0.1 g/l; $(NH_4)_6Mo_7O_{24}$ $4H_2O$, 0.0002 g/l; H_3BO_3, 0.0005 g/l; $ZnCl_2$, 0.0003 g/l; $CoCl_2$ $6H_2O$, 0.00003 g/l; $FeCl_2$ $6H_2O$, 0.0002 g/l; pH7.0, glucose (1.0 g/l) was supplemented to MM when endosulfan used as the sole sulfur source (NSM). The minimal salt medium (MSM) had the following composition (KH_2PO_4, 1.0 g/l; K_2HPO_4, 1.0 g/l; NH_4NO_3, 1.0 g/l; $MgSO_4$. $7H_2O$, 0.2 g/l; $CaCO_3$, 0.02 g/l, $FeSO_4$, 0.01 g/l; 0.02 g/l, $CaCl_2$; pH 6.5) containing endosulfan as the sole carbon and energy source. Bacto agar was added to the above media at a concentration of 1.5% (w/v) to prepare solid media. Endosulfan was dissolved in acetone as stock solutions (5×10^4 mg/l), Which was rationed into medium to get the desired concentrations [2,13].

2.2. Isolation of endosulfan degrading bacteria by enrichment and screening

Activated sludge samples (the endosulfan-contaminated) were collected from a wastewater treatment facility in pesticide manufacturers of Jiangsu, China. Samples (10 ml) were suspended in 90 ml TYC media containing 50 mg/l endosulfan in a 250-ml Erlenmeyer flask, and incubated at 30 °C with shaking (150 rpm). After 7 days, 10 ml of each culture was reinoculated into new TYC media while the endosulfan concentration increased doubly and further incubated at 30 °C for 7 days. After the fifth enrichment transfer, each culture (10 ml) was centrifuged to get the pellets and washed twice with 0.1 M phosphate buffer (pH 7.0). Then the cell was used to inoculate in separate flasks containing 100 ml of MM with endosulfan (300 mg/l); and then further screened weekly by endosulfan. After the fourth screening transfer, an aliquot (0.2 ml) from each culture was applied to solid MM (containing 1.5% agar and 300 mg/l of endosulfan) for isolation of single colonies. Isolates were purified further by streaking on fresh plates. A single colony was transferred to liquid acetone-MM, respectively, to exclude acetone dependent strains.

2.3. Identification of isolated endosulfan degrader

The isolate was characterized by ATB Expression System (Biomerieux, France) and by the 16S rDNA sequence analysis. Genomic DNA was extracted as described previously [26]. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) [27]. PCR products were cloned into a pMD18-T vector (TaKaRa) and sequenced. The 16S rRNA partial sequence of strain CS5 was deposited in the GenBank database under accession number EU266588.

2.4. Biodegradation of endosulfan in aqueous medium

Strain CS5 was pre-cultured in 250 ml flasks containing 100 ml TYC media with supplement of 100 mg/l endosulfan. After 2 days, cells were collected by centrifugation, and were washed twice with 0.1 M phosphate buffer (pH 7.0). The strain was reinoculated in 50 ml MM, NSM and MSM, which resulted in an optical density of 0.2 at 600 nm. All of media were supplemented with endosulfan (50 mg/l) and incubated under aerobic conditions in a rotatory shaker (150 rpm) at 30 °C for 8 days. Uninoculated media with the same concentration of endosulfan were used as controls. The residue of endosulfan and its metabolites, culture pH and biomass were determined at 1-day interval. The biomasses of microorganisms were regularly checked by a spectrophotometer (SCINCO S-3100, Korea) at 600 nm. The pH was measured using a pH meter (PHS-3D, REX, China). The extracted samples were filtered through a 0.25-μm filter before being analyzed by High Performance Liquid Chromatography (HPLC, Thermo Finnigan, USA) as described previously with modifications [28]. The analytical column was Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm), The solutes were detected using PDA detector with gradient UV–vis detection ranging from 200 to 600 nm. A mixture of acetonitrile and water (80:20, v/v) was used as the mobile phase at a flow rate of 1.0 ml/min. The injection volumes were 20 μl.

2.5. Enzymatic degradation by cell-free extracts

Enzymatic degradation of endosulfan by cell-free extracts was tested as described [29]. Bacterial cells grew in TYC media with or without endosulfan were harvested at mid-growth phase by centrifugation at 6000 × *g* for 10 min at 4 °C. The bacterial pellets were washed twice with phosphate buffer (0.05 M, pH 7.0) and then suspended in 3 ml of phosphate buffer. The ultrasonication was performed at 200 W for nine 5-s bursts with a 5-s cooling period in ice water in between bursts. After disruption of cells by ultrasonication and centrifugation at 4 °C, the supernatants were obtained as the cell-free extracts (protein concentration was diluted to 1 μg/μl with phosphate buffer before use). The reaction mixture (1.0 ml) contained phosphate buffer (0.05 M, pH 7.0), endosulfan (50 mg/l) and cell-free extract. Reactions were performed at different pH (5–8), temperatures (20–50 °C), and the residues of endosulfan were quantified by HPLC as described above. The cultures with the same concentration of endosulfan but with no cell-free extract were used as controls. All experiments were performed in triplicate.

2.6. Biodegradation of endosulfan in soil

Biodegradation of endosulfan in soil by *Achromobacter xylosoxidans* CS5 was tested as described by Xing Huang et al. [30]. Soil samples were collected from the top 0–10 cm from the agricultural farm at Taian, China. This soil had characteristics of a sandy loam with 70% sand, 16% silt and 8% clay, 6% organic matter, and a pH of 6.9. The soil has never been treated with endosulfan. Soil samples were dried at room temperature, and then sieved to 5 mm and stored at 4 °C. Soil samples were sterilized. The solution of endosulfan was added into the samples (100 g) fresh soil and the sterile soil to give the concentration of 50 mg/kg, and mixed well. One set of fresh soil and sterile soil were inoculated with strain CS5 (OD_{600} = 0.3 = 10^6 cells/g of soil^{-1}). Another set of uninoculated soil

was kept as a control. The inoculum was thoroughly mixed into the soils under sterile conditions, and the moisture was adjusted to 65% (w/w of dry weight of soil). Each soil microcosm was incubated under aerobic conditions at 30 °C in the dark. 10 g of the soil samples were removed at regular intervals of 0, 6, 12, 24 and 30 d, and the endosulfan and its metabolites were analyzed.

2.7. Assay of endosulfan and its metabolites by HPLC

Endosulfan and its metabolites in cultures were extracted by addition of equal volume of acetonitrile in a whole flask and shaken for 1 h with reciprocating shaker and then centrifuged. For endosulfan and its metabolites extraction from soil, 10 g of soil sample

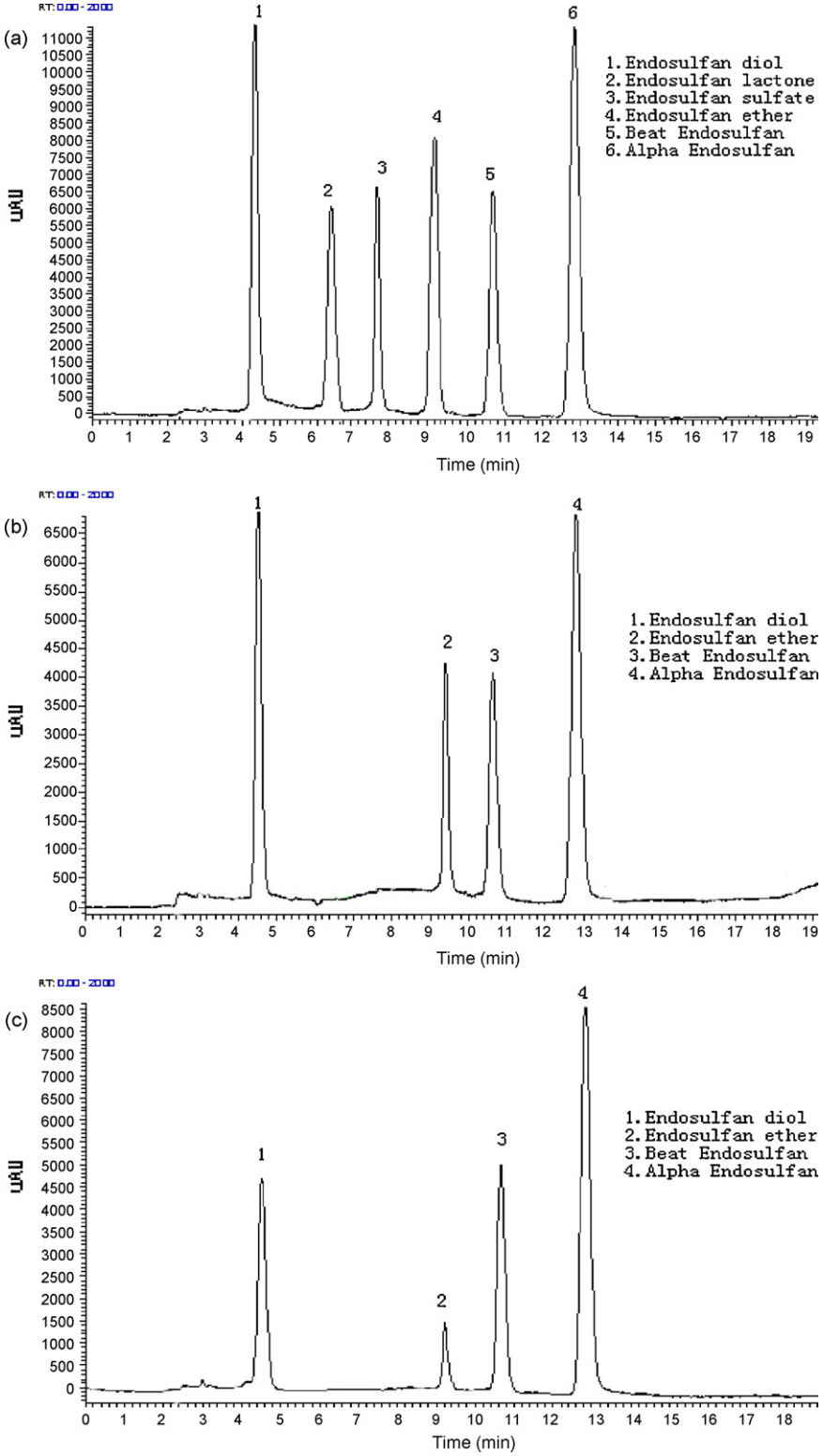

Fig. 1. (a) The HPLC chromatograms of endosulfan and its metabolites at standard conditions; (b) the HPLC chromatograms of endosulfan and its major metabolites in aqueous medium; (c) the HPLC chromatograms of endosulfan and its major metabolites in soil.

was extracted with 50 ml acetonitrile, the mixture was shaken for 1 h at 200 rpm on a rotary shaker and then centrifuged. The supernatant was decanted into a glass bottle, and the organic solvent was concentrated in a water bath at 35 °C. The extracted samples were detected by the HPLC. The compounds were identified by comparison of HPLC retention times to those of authentic standards. Retention times for α-endosulfan, β-endosulfan, endosulfan sulfate, endosulfan ether, endosulfan diol and endosulfan lactone under these analytical conditions were 12.57, 10.43, 7.58, 9.15, 4.21 and 6.42 min respectively (Fig. 1a). The recovery of endosulfan from liquid culture was at between 97% and 100%. Extraction efficiency of endosulfan from soil was always found to be between 88% and 96%. All experiments were performed in triplicate.

2.8. Genotoxicity study

The genotoxicities study of the residual endosulfan and the formed metabolites in aqueous medium and soil were carried out by the *umu*-test [31]. *Salmonella typhimurium* TA1535/pSK1002 in the *umu*-test carrying an *umuC-lacZ* fusion gene on multicopy plasmid pSK1002 was a kind gift from Dr. Y. Oda (Osaka Prefectural Institute of Public Health, Japan). The residual endosulfan and its metabolites in aqueous medium and soil were extracted with acetonitrile as described above. The *umu*-test was performed in general by the method of Oda et al. [20]. Briefly, the bacterial cells were cultivated overnight at 37 °C in Luria–Bertani broth containing ampicillin (25 mg/l). The culture was diluted 50-fold with TGA medium by adding ampicillin (20 mg/l) and further incubated at 37 °C until the bacterial OD_{600} reached about 0.3. The cultures were subdivided into 1.7 ml aliquots in the test tubes, to a test chemical (20 μl) were added, acetonitrile was used as negative control. These mixtures were incubated at 37 °C for 2 h with vigorous shaking (at 155 rpm), and then the bacterial density and the β-galactosidase activity were measured by the method of Miller with slight modification as described by Oda et al. [20,32]. In the *umu*-test, a sample is supposed to be genotoxic, if the induction rates of the system, indicated by the β-galactosidase activity, exceeds 2.0 [20]. All experiments were performed in triplicate. The result was calculated as an induction ratio related to β-galactosidase activity:

$$\text{Induction ratio} = \frac{\beta\text{-galactosidase activity of sample}}{\beta\text{-galactosidase activity of negative control}}.$$

3. Results

3.1. Isolation and characterization of endosulfan degrading bacteria

From the activated sludge samples, 10 different bacteria were isolated which were able to use endosulfan as the sole carbon, sulfur and energy source at high concentration of endosulfan (300 mg/l). Among the different ten strains, nine strains showed active growth in acetone-MM medium. However, strain CS5 had very poor growth in acetone-MM and finally selected as an endosulfan degrader. Strain CS5 was Gram-negative and oxidase-positive. The ATB Expression System identified the strain as *A. xylosoxidans* at 99% confidence. The 16S rRNA sequence of strain CS5 showed greatest similarity to the reference sequences from members of the *A. xylosoxidans* within the GenBank database.

3.2. Biodegradation of endosulfan in aqueous medium

A. xylosoxidans CS5 was cultivated in MM, NSM and MSM for 8 days. After 8 days, 24.8 mg/l α-endosulfan and 10.5 mg/l β-endosulfan were removed by strain CS5 in MM when endosulfan

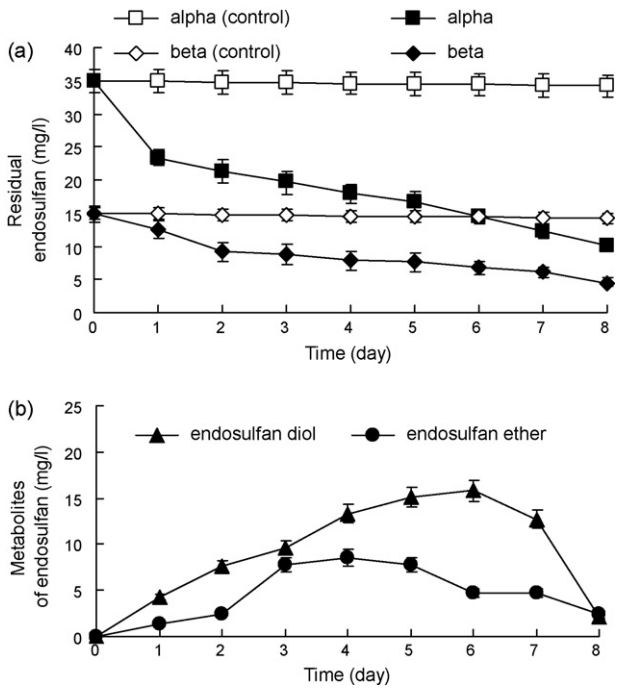

Fig. 2. (a) Biodegradation of endosulfan in MM by *Achromobacter xylosoxidans* strain CS5; (b) the formation of major metabolites of endosulfan in MM by *A. xylosoxidans* strain CS5 (means and standard deviations of triplicates are shown).

was used as the sole carbon and sulfur source (Fig. 2a). However, more endosulfan biodegradation was observed in NSM and MSM. It could degrade 30.8 mg/l and 31.6 mg/l α-endosulfan when endosulfan was used as the sole sulfur source in NSM and as the sole carbon source in MSM, which was significantly higher than that recorded in MM (Figs. 3a and 4a). Similarly, 13.65 mg/l and 13.8 mg/l β-endosulfan were degraded in NSM and MSM, respectively. In controls without incubation, abiotic degradation was negligible throughout all studies. All degradation was accompa-

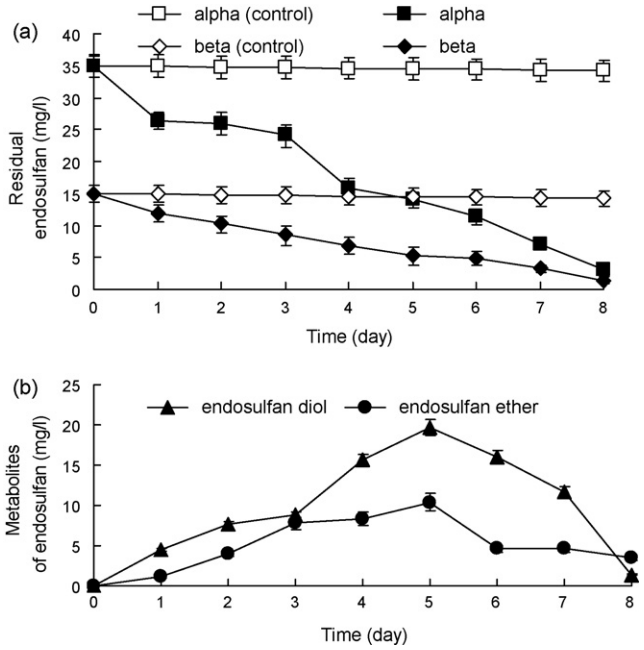

Fig. 3. (a) Biodegradation of endosulfan in NSM by *Achromobacter xylosoxidans* strain CS5; (b) the formation of major metabolites of endosulfan in NSM by *A. xylosoxidans* strain CS5 (means and standard deviations of triplicates are shown).

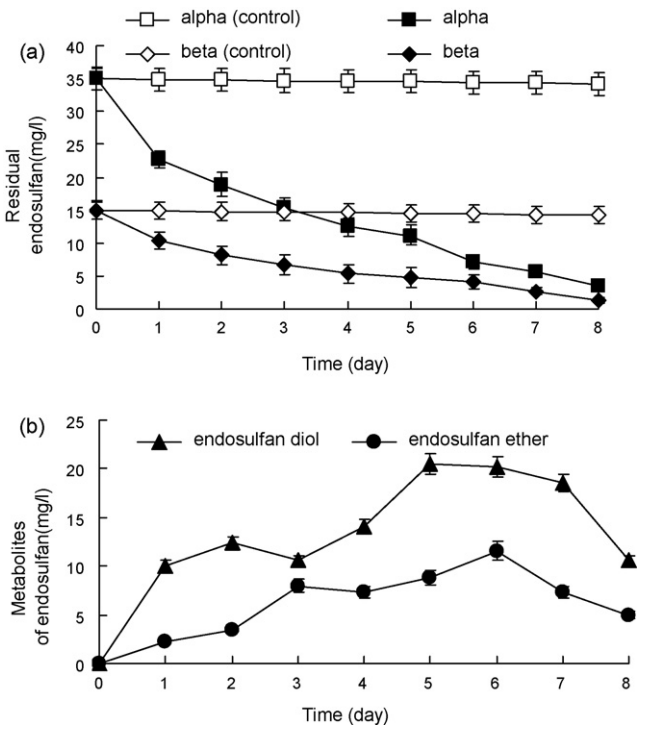

Fig. 4. (a) Biodegradation of endosulfan in MSM by *Achromobacter xylosoxidans* strain CS5; (b) the formation of major metabolites of endosulfan in MSM by *A. xylosoxidans* strain CS5 (means and standard deviations of triplicates are shown).

nied by bacterial growth as shown in Fig. 4. The cultures pH was slightly decreased in MM and NSM, but the pH in MSM rapidly decreased from 6.5 to 4.7 in 1 day, and slightly increased thereafter (Fig. 5).

3.3. Enzymatic degradation endosulfan by cell-free extracts of strain CS5

Endosulfan was degraded rapidly in 1 h by the cell-free extracts (Fig. 6). 90.8% (31.8 mg/l) of the α-endosulfan and 89.2% (13.4 mg/l) β-endosulfan were degraded by cell-free extracts with induction, respectively. Similarly, 89.8% (31.4 mg/l) of the α-endosulfan and 87.6% (13.1 mg/l) β-endosulfan were degraded by cell-free extracts without induction, respectively. In controls without cell-free extracts, the degradation was negligible. The bacterial cell-free extract was able to degrade endosulfan at the temperatures ranging from 20 to 50 °C and the most rapid degradation was observed

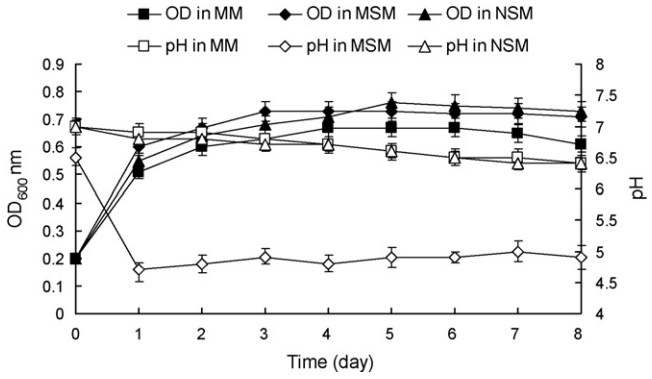

Fig. 5. Bacterial growth monitored by measuring optical densities at 600 nm and culture pH in media in 8 days (means and standard deviations of triplicates are shown).

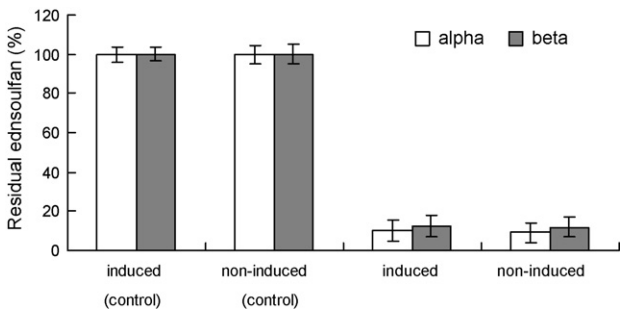

Fig. 6. Enzymatic degradation of endosulfan by cell-free extract of *Achromobacter xylosoxidans* strain CS5 (means and standard deviations of triplicates are shown).

at 40 °C (Fig. 7a). It was capable of degrading endosulfan in the pH ranging from 5 to 8, with the most rapid degradation rates at pH 6.0 (Fig. 7b). The enzymatic degradation rate specific for α-endosulfan and β-endosulfan were about 13.6 mg/l/h and 31.2 mg/l/h, respectively. Endosulfan diol as a major metabolite was detected in this study, and also a small quantity of endosulfan ether was formed (data not shown). These results show that endosulfan was transformed by soluble enzymes from cell-free extracts of strain CS5.

3.4. Biodegradation of endosulfan in soil

The degradation patterns of endosulfan in soil are shown in Fig. 8. Only 10.9 mg/l α-endosulfan and 3.9 mg/l β-endosulfan were removed in uninoculated fresh soil after 30 days of incubation. In contrast, 28.9 mg/l α-endosulfan and 12.4 mg/l β-endosulfan were removed after 30 days for fresh soil inoculated with strain CS5 (Fig. 8a). Therefore, endosulfan removal improved significantly in soils when strain CS5 was inoculated. Simultaneity, 25.8 mg/l α-endosulfan and 11.2 mg/l β-endosulfan were removed for sterile

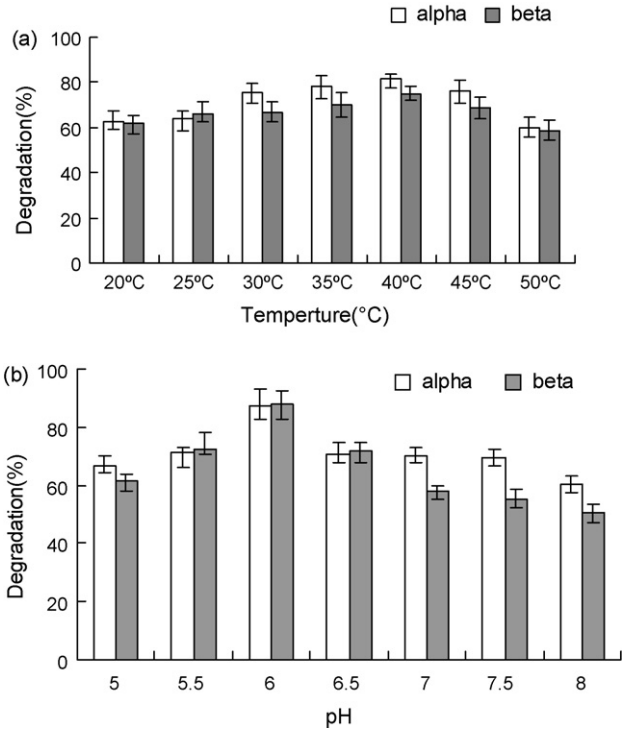

Fig. 7. (a) Enzymatic degradation study of endosulfan under different temperatures (20–50 °C); (b) enzymatic degradation study of endosulfan under different pH (5–8) (means and standard deviations of triplicates are shown).

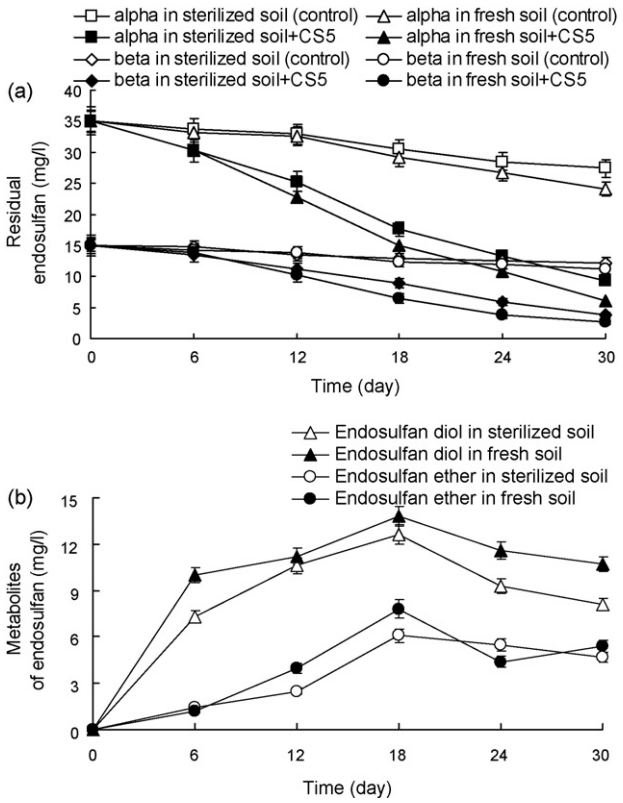

Fig. 8. (a) Removal of endosulfan by *Achromobacter xylosoxidans* strain CS5 in soil; (b) the formation of major metabolites of endosulfan by *A. xylosoxidans* strain CS5 in soil (means and standard deviations of triplicates are shown).

soil when strain CS5 was inoculated. Similarly, the removal in fresh uninoculated soil was superior to that in the uninoculated sterile soil.

3.5. Analysis of endosulfan biodegradation metabolites

The metabolites formed were analyzed by HPLC. Endosulfan diol and endosulfan ether were detected as the major metabolites after comparing with the authentic standards (Figs. 1b,c, 2b, 3b, 4b and 8b). The concentration of endosulfan diol increased and reached a maximum (15.8 mg/l in MM, 19.7 mg/l in NSM, 20.5 mg/l in MSM), and was decreased thereafter. Endosulfan ether showed a similar pattern, but its highest concentrations were 8.5 mg/l in MM, 10.4 mg/l in NSM and 11.6 mg/l in MSM, respectively (Figs. 2b, 3b and 4b). The endosulfan diol and endsulfan ether were not accumulated and degraded in culture subsequently. Similarly, endosulfan diol and endosulfan ether were detected as major metabolites in soil (Figs. 1c and 8b). The concentration of endosulfan diol and endsulfan ether reached a maximum of 13.8 mg/l and 7.8 mg/l in inoculated fresh soil after 18 days of incubation. Other metabolites such as endosulfan sulfate were not found in the cultures.

3.6. Genotoxicity studies

In the biodegradation process, the potential genotoxicities of residual endosulfan and the formed metabolites in aqueous medium and soil were evaluated using *Salmonella typhimurium* TA1535/pSK1002. As shown in Fig. 9, endosulfan is the genotoxic compound, because the induction ratios of the samples with uninoculated media and 0 day samples were about 3.5. However, in decomposition processes, the degradation of endosulfan was

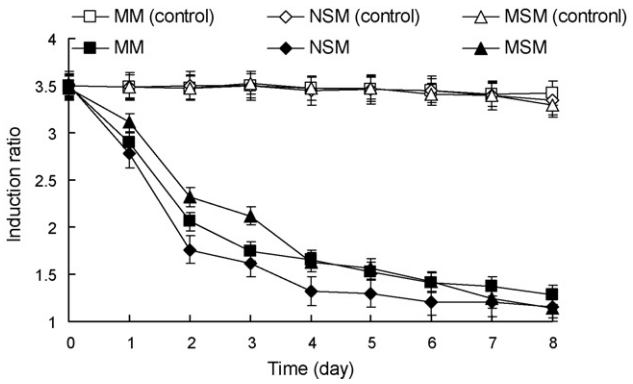

Fig. 9. Genotoxicity of endosulfan in aqueous medium in the 8 days biodegradation process (means and standard deviations of triplicates are shown).

accompanied by the significant decrease and final disappearance of genotoxicity. The induction ratios decreased from 3.5 to 1.28 in MM, from 3.47 to 1.14 in NSM and from 3.47 to 1.15 in MSM after 8 days, respectively. In controls without incubation, genotoxicity decrease was negligible throughout all studies. Similarly, the degradation of endosulfan was accompanied by the significant decrease in soil (Fig. 10). The induction ratios decreased from 4.2 to 1.82 in sterile soil, from 4.29 to 1.73 in fresh soil after 30 days, respectively.

4. Discussion

Strain CS5 could utilize endosulfan as the sole carbon, sulfur and energy source and was identified as *A. xylosoxidans*. To our knowledge, this is the first report on endosulfan biodegradation by *A. xylosoxidans*. The bacterium has been previously documented as excellent degrader of aromatic hydrocarbons and xenobiotics both in soil and water environment. *A. xylosoxidans* strain B-16 could grow on bisphenol A as a sole carbon source under aerobic condition [33]. The bacterium *A. xylosoxidans* subsp. *denitrificans* strain EST4002 could degrade 2,4-dichlorophenoxyacetic acid (2, 4-D) [34]. Therefore, these findings support the role of the *A. xylosoxidans* in bioremediation of endosulfan.

Previous researchers have reported endosulfan as a sole sulfur source [1,11,35], or as a sole carbon source for microbial growth [13–17]. In this study, *A. xylosoxidans* CS5 could utilize endosulfan as the sole carbon, sulfur and energy source. Degradation tests were investigated when endosulfan was used as a carbon or sulfur source. Results indicated that strain CS5 could degrade nearly equal amount of endosulfan when it was used as the sole sulfur source or as the sole carbon source. When endosulfan was used as

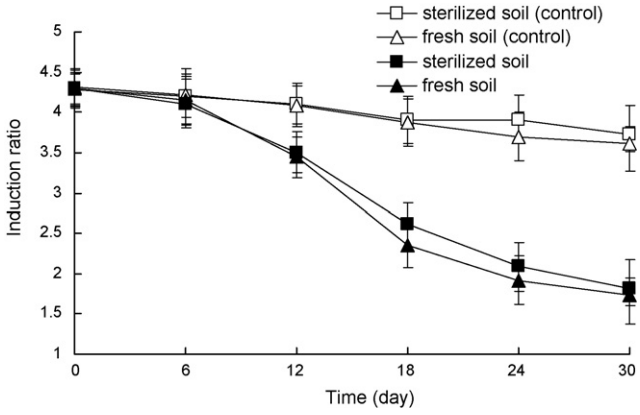

Fig. 10. Genotoxicity of soil with the endosulfan in the 30 days biodegradation process (means and standard deviations of triplicates are shown).

the sole carbon, sulfur and carbon source, however, the degradation rate was lower than that of endosulfan was used as the sole carbon source or as the sole sulfur source. This is most likely attributed to the fact that when strain was cultured in NSM and MSM, it showed higher bacterial densities (Fig. 5). Strain CS5 degraded the high amount of α- and β-endosulfan in the NSM and MSM, producing the highest bacterial densities as compared which in MM. Lower microbial population in MM may be due to the presence of low nutrient.

It is generally accepted that culture pH is related to endosulfan metabolites [17]. Therefore, the pH of the MM drastically decreased to acidic range due to metabolic activities with simultaneous degradation of endosulfan and the formation of major metabolites. The results confirmed the findings of previous studies [10,11,16–18]. They reported that decrease in pH might be due to the formation of HCl or organic acids by microorganisms. There was slightly pH decrease in MM and NSM in this study. We speculated that the contents of K_2HPO_4 was too high and it neutralized HCl and organic acids. The non-accumulation of endosulfan diol and endosulfan ether in liquid cultures formed indicated that both metabolites were transient intermediates, there was an initial hydrolysis of endosulfan that resulted in the formation of an intermediate metabolite, endosulfan diol, which is further metabolized to endosulfan ether [2,11,17,36]. These results revealed that these bacterial species adopted to the hydrolytic pathway of endosulfan biodegradation [11,13,14].

In the cell-free system, endosulfan could interact directly with target proteins, which was contrast to contrast to the cell-living system where endosulfan should be first carried over the membranes to be metabolized. Therefore, endosulfan could be rapidly degraded in 1 h. When the cell-free degradation reactions were performed at temperatures ranging from 20 to 50 °C and in the pH ranging from 5 to 8, α-endosulfan and β-endosulfan ranging from 50.5% to 90.8% were rapidly degraded in 1 h which suggested that the target proteins could suite to a wide temperature and pH. Moreover, cell-free systems made of proteins from bacteria grown with or without endosulfan showed the same degradation profile. Therefore, the endosulfan degradation enzyme was constitutively expressed in the cell. Further researches on isolation and identification the relevant enzymes and genes from strain CS5 are necessary.

Successful removal of endosulfan from soil by implanted bacteria has been previously reported [22–24]. It is reported that endosulfan was effectively biodegraded miniature and bench scale soil reactors [22]. *Pseudomonas aeruginosa* removed more than 85% of spiked α-endosulfan and β-endosulfan (100 mg/l) after 16 days in loam soil [24]. In this study, the addition of strain CS5 to soil treated with endosulfan resulted in a higher removal rate than that observed in uninoculated soils. Similarly, endosulfan removal was slightly better in fresh soil inoculated with strain CS5 than in inoculated sterilized soil suggesting a contribution of the indigenous flora to endosulfan removal.

The *umu*-test is a sensitive tool for the detection of genotoxicity of different origins and in different mixtures [37]. In the *umu*-test, it was observed that the metabolism of endosulfan isomers was accompanied by a substantial reduction in their genotoxicity to the test bacteria. The induction rates was higher in soil than in aqueous medium, we speculated that there were others genotoxic compound in soil. Previous researchers reported the reduction in toxicity of endosulfan by the bacterial culture [13,14,16]. In present study, the genotoxicity of the residual endosulfan and its metabolites was negative to the test bacterial after degradation. The findings suggest that the isolated bacterial culture mediates the metabolism of α-endosulfan and β-endosulfan, and the formed metabolites are less toxic. These results reflect the strain CS5 has the potential for the safe treatment of endosulfan contaminated water and soil.

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