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Biodegradation of Endosulfan by Bacterial Strain *Alcaligenes faecalis* JBW4 in Argi-Udic Ferrosols and Hapli-Udic Isohumosols

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Abstract The overapplication of endosulfan on crops has resulted in the widespread contamination of soil. In this study, we examine the potential for bioremediation of the bacteria strain *Alcaligenes faecalis* JBW4 in degrading endsosulfan in soils. Bacteria were inoculated into sterilized and non-sterilized soils (Argi-Udic Ferrosols and Hapli-Udic Isohumosols) spiked with endosulfan. The results obtained from polymerase chain reaction-denaturing gradient gel electrophoresis indicate that JBW4 colonized Argi-Udic Ferrosols and Hapli-Udic Isohumosols successfully. The degradation efficiencies of α and β isomers of endosulfan by JBW4 were higher in Hapli-Udic Isohumosols than in Argi-Udic Ferrosols, and α and β isomers were degraded by 100.0 and 69.8%, respectively. In addition, detected endosulfan metabolites were either endosulfan ether and endosulfan lactone. Results of the single-cell gel electrophoresis assay showed that the toxicity of endosulfan and its metabolites in Hapli-Udic Isohumosols decreased after 77 days when compared to those in Argi-Udic Ferrosols after degradation by JBW4. Strain JBW4 is an excellent bioremediator through its ability to degrade endosulfan in contaminated Argi-Udic Ferrosols and Hapli-Udic Isohumosols.

1 Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9 a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide, CAS No. 115-29-7) is one of the most widely used organochlorine pesticides globally (Singh and Singh 2014). As a commercial product, it is a mixture of two stereo isomers: α- and β-endosulfan in the range of 67–72% and 28–32%, respectively (Thangadurai and Suresh 2014). For five decades, it has been manufactured and used to control insects in a variety of crops in several countries (Poolpak et al. 2008; Jia et al. 2009). The dire and chronic health risks of endosulfan is well known, with acute affects in aquatic organisms, animal species, and humans at relatively low exposure levels (Wan et al. 2005; Svartz et al. 2014). Moreover, endosulfan is listed as one of the most persistent organic pollutants in 2011 (Chakrabarty et al. 2012). Although bans and regulations were imposed on the production and usage of endosulfan in many countries, it is still available as an insecticide in China (Zhao et al. 2014). In addition, endosulfan residue has been detected in soils in many crop production areas

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of China due to its high persistence (Jia et al. 2010; Castillo et al. 2011; Tao et al. 2013).

There are several methods for removing endosulfan, including adsorption, filtration, and chemical oxidation (or) reduction, all of which have their own limitations (Thangadurai and Suresh 2014). However, microbial degradation presents an efficient, environmentally sound, and cost effective alternative approach to remediate contaminated environments (Kataoka and Takagi 2013; Nikolic et al. 2014). In a previous publication, an *Alcaligenes faecalis* strain, JBW4, that was capable of degrading endosulfan, was isolated by researchers in our laboratory (Kong et al. 2013a). However, the biodegradation using the isolated degrading-bacteria in soils is still inadequate (Kataoka and Takagi 2013) and is affected by factors such as pH, organic matter content, and bioavailability of pesticides (Phillips et al. 2005; Kataoka and Takagi 2013). Different soils have different physical, chemical, and biological properties (Girvan et al. 2003), which may influence the degradation rate of endosulfan and the formation of endosulfan metabolites following degradation (Parkpian et al. 1998; Varon-Lopez et al. 2014). Kumar and Philip (2006) studied the adsorption and desorption characteristics of endosulfan in four types of Indian soils; however, the biodegradation of endosulfan was not investigated. Kong et al. (2013b) studied the degradation of endosulfan by JBW4 in brown soils: the pH value and organic matter content in brown soils are 7.6 and 17.6 g/kg, respectively. In order to comprehensively understand potential of JBW4 in degrading endosulfan, it is essential to study the biodegradation by strain JBW4 in a variety of soil types contaminated with endosulfan. Argi-Udic Ferrosols are distributed over Fujian and other provinces in the south of China. The pH value and organic matter content are 5.22 and 9.62 g/kg, respectively. Hapli-Udic Isohumosols are distributed mainly over the northeast of China. The pH value and the content of organic matter are 7.21 and 23.46 g/kg, respectively. Because of these values, Argi-Udic Ferrosols (AUFs) and Hapli-Udic Isohumosols (HUIs) were chosen from the many types of soil in China as the two representative experimental soils in this study.

There are several proposed pathways of endosulfan biotransformation by soil bacteria. One of the pathways results in the formation of endosulfan diol which is nontoxic as compared to endosulfan by hydrolysis (Kullman and Matsumura 1996). One of the pathways resulted in the formation of endosulfan sulfate by oxygenation (Singh and Singh 2011). Endosulfan sulfate has a similar toxicity and persistence compared to the original compound (Antonious and Byers 1997). It is thereby necessary to detect specific endosulfan metabolites in determining the associated metabolic pathways and toxicity after degradation in HUIs and AUFs. Single-cell gel electrophoresis (SCGE) was used to assess the toxicity of endosulfan and its metabolites, as SCGE was reported to be sensitive to low endosulfan concentrations (Kong et al. 2013b).

Other studies have previously reported the biodegradation of endosulfan or endosulfan sulfate in the soil or liquid phase by native microbes or the addition of microbes (Parkpian et al. 1998; Fuentes et al. 2011; Thangadurai and Suresh 2014). This research focused on the degradation rates of endosulfan and metabolites. However, less attention has been given to the survival of the introduced microbial population in soil or liquid phase. One of the primary objectives of this study was to investigate the growth of JBW4 during endosulfan biodegradation. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to study the survival of strain JBW4 in soils by Kong et al. (2013b). PCR-DGGE has seen wide use in determining the presence and abundance of microbial communities in several research fields, including bioremediation (Varon-Lopez et al. 2014; Zhang et al. 2015). Thus, the PCR-DGGE technique was employed to investigate the ability of JBW4 to colonize soils in this study.

The objectives of this study were to: (a) assess the degradation and decontamination ability of strain JBW4 in endosulfan-contaminated AUFs and HUIs, (b) identify endosulfan metabolites and propose the metabolic pathways by which endosulfan may be metabolized in the two soils (AUFs and HUIs), (c) study JBW4 colonization during endosulfan degradation in the two soils (AUFs and HUIs), and (d) study the changes in toxicity of endosulfan and its metabolites in the two soils following degradation.

2 Materials and Methods

2.1 Chemicals and Reagents

Analytical-grade endosulfan (99.7% purity), endosulfan diol (99.4% purity), endosulfan ether (99.0% purity), endosulfan sulfate (97.7% purity), and endosulfan lactone (99.0% purity) were purchased from Dr.

Ehrenstorfer GmbH, Augsburg, Germany. All other reagents used in this study were of analytical grade. Prior to use, all of the organic solvents were redistilled.

2.2 Soil Sample Information and Preparation of Soil Inoculants

The AUFs and HUIs were selected for the biodegradation experiments. The chemical and physical properties of the two soils were shown in Table 1. And the analysis for measuring plant available nutrients was conducted by conventional standard procedures (Lu 2000). The soil pH was determined at a soil to water ratio of 1:2.5. The AUFs were collected from Wuyishan city (27.55 °N, 118.05 °E) of Fujian province, China. The HUIs samples were collected from Qiqihaer city (47.33 °N, 123.97 °E) in Heilongjiang province, China. Additionally, endosulfan residue was below the detection limit in the two soils. Soil samples were collected from the top 10–30 cm. Strain JBW4 capable of utilizing endosulfan as a carbon and energy source was isolated from activated sludge from a company that produced endosulfan (Kong et al. 2013a). It is deposited in the China Center for Type Culture Collection with the strain number M 2012181 (Kong et al. 2013a). The microbial inoculant for the studies was prepared using pure cultures of JBW4 in 100 mL LB nutrient medium in Erlenmeyer flasks at 30 °C with continuous shaking at 160 rpm for 24 h. After incubation, the medium was centrifuged (Eppendorf, Centrifuge 5804). The supernatant was removed from the Eppendorf tubes, and the pellet cells were adjusted to a set optical density ($OD_{600} = 0.5$) with sterilized water for biodegradation studies.

2.3 Soil Microcosm Experiment

Soil samples were sieved (<0.5 mm) and activated by incubation at 25 °C for 72 h prior to use. Approximately 20 g of non-sterilized soil and sterilized soil sample were added to the reagent bottles, respectively. The sterilized soil samples were autoclaved fully for 60 min at 121 °C and were studied under aseptic conditions in an asepsis room over the whole experimental period. The endosulfan stock solution was prepared in acetone. The bottle was spiked with 0.5 mL endosulfan (2000 mg/L) to receive an initial concentration of 50 mg endosulfan per kilogram of soil. C_0 is the initial concentration of endosulfan that is determined in 0 day. The contaminated soil was inoculated with bacterial inoculant at a concentration of 2×10^8 cfu/g soil (Kong et al. 2013b). Deionized water was added to the soil samples each day to compensate for the loss of water due to evaporation. The sterilized water was also added to the sterilized soil under the aseptic conditions. Five treatments were as follows: CK—non-sterilized soil, SE—sterilized soil with endosulfan, SEJ—sterilized soil with endosulfan and JBW4, NE—non-sterilized soil with endosulfan, and NEJ—non-sterilized soil with endosulfan and JBW4. These tests were conducted in triplicate. The samples were collected from bottles of each treatment at 0, 3, 7, 14, 21, 28, 35, 49, 63, and 77 days and analyzed for concentrations of endosulfan isomers. At 3, 5, and 7 days, samples were collected and analyzed for the colonization of JBW4. At 3, 21, 49, and 77 days, samples were collected and tested for the toxicity of soil samples. At 77 days, samples in the NEJ treatment were collected and tested for the endosulfan metabolites.

2.4 Extraction Procedure and Determination of Endosulfan

The extraction procedure and endosulfan determination method were performed as described previously by Kong et al. (2013b). Briefly, a soil sample (20 g) spiked with endosulfan was extracted in acetone, and purified, followed by GC analysis for detection.

Table 1 Soil chemical and physical properties

Soil type	Organic matter (g/kg)	Available potassium (mg/kg)	Available nitrogen	Available phosphorus	Water holding capacity (%)	pH	Silt (%)	Sand (%)	Clay (%)
Argi-Udic Ferrosols	9.62	66.34	44.22	8.32	20.42	5.22	37.95	44.62	17.43
Hapli-Udic Isohumosols	23.46	136.27	129.36	29.61	29.57	7.21	29.03	42.25	28.72

2.5 Assay of Endosulfan Metabolites

The analysis of metabolites produced by the degradation of endosulfan at 77 days was performed as described previously by Kong et al. (2013b) with some changes. The samples were tested by gas chromatography-mass spectrometry (GC-MS) (PE Clarus 500) using the same extraction method that was used for the GC analysis experiment. High-purity helium was used as carrier gas at a flow rate of 1.1 mL/min. The injector port temperature was 250 °C and the column temperature was set to an initial temperature of 100 °C (hold 1 min), increased to 280 °C at a heating rate of 10 °C/min (hold 6 min). The transfer line temperature was set at 250 °C. The electron ionization was performed with a source temperature of 200 °C.

2.6 Colonization of JBW4

DNA extraction from soil samples and colonization of JBW4 using DGGE was conducted as described previously by Kong et al. (2013b) without modification. Briefly, total DNA of the sample was extracted using E.Z.N.Z.^{TM} Soil DNA Kit following manufacturer's instructions. Then, the obtained DNA was used as the template for nested PCR to amplify 16s rDNA genes. The variable V3 region was amplified using the obtained 16s rDNA genes as the template. The finally obtained PCR products were analyzed by DGGE.

2.7 Measurement of Toxicity of Endosulfan and its Metabolites Using the Comet Assay

In the present study, the SCGE was conducted to evaluate the toxicity in earthworm coelomocytes induced by endosulfan and its metabolites after degradation. The OTM was the distance between the center of the head and the center of the tail and the percentage of total DNA in the tail. DNA damage tests in earthworm cells were conducted using the method described previously by Kong et al. (2013b) without modifications. Briefly, the soil samples were extracted for conducting the toxicological tests on earthworms (*Eisenia fetida*). After exposing the earthworms to the extract, the coelomocytes of the earthworms were prepared by the non-invasive method followed by the SCGE.

3 Results and Discussion

3.1 Fortified Recovery of Endosulfan from Soil

To determine method efficiency, recovery experiments of three concentrations (5, 25, 50 mg/kg) in AUFs and HUIs spiked with endosulfan were carried out. The recovery ranges of α-endosulfan and β-endosulfan were 90.27~102.1% and 94.25~98.56%, respectively. The coefficients of variation were 1.5~2.4% and 0.8~2.6% for α-endosulfan and β-endosulfan, respectively.

3.2 Degradation of Endosulfan in AUFs and HUIs

Time course for degradation of both endosulfan isomers and the calculated half-life of degradation are shown in Fig. 1 and Table 2. As depicted in Fig. 1a, the concentrations of α-endosulfan in SE and NE treatments of AUFs always kept a higher level than those in SEJ and NEJ treatments inoculated with degrading-bacteria. After the 77 days, the degradation rate of α-endosulfan in AUFs varied as the following: NEJ > SEJ > NE > SE in different treatments. There were similar results about the degradation of β-endosulfan in AUFs (Fig. 1b) and that of both endosulfan isomers in HUIs (Fig. 1c, d). These results indicated that degrading-bacteria JBW4 had the ability to accelerate the degradation of both endosulfan isomers in the two soils.

The biotic degradation can be described using a first degradation curve: $C = C_0 \cdot \exp(-kt)$ where C is the concentration of the endosulfan isomers at time t and C_0 is the initial concentration of endosulfan. First-order kinetic plots are used to determine the rate constants (k) and correlation coefficient (R^2) (Table 2). In AUFs, the half-life of α-endosulfan in the soil of NE, NEJ, SE, and SEJ treatments were 111.9, 32.4, 129.3, and 42.1 days, respectively. The half-life of β-endosulfan in the soil of NE, NEJ, SE, and SEJ treatments was 162.6, 50.4, 175.0, and 65.7 days, respectively. Thus, the half-life of both endosulfan isomers in NEJ treatment was significantly shorter than those in NE treatment. Meanwhile, the half-life of both endosulfan isomers in SEJ treatment was considerably shorter than those in SE treatment. Thus, strain JBW4 promoted the degradation and shortened the half-live of both endosulfan isomers dramatically in unsterilized soils as well as sterilized soils of AUFs. Strain JBW4 also had the similar positive influence on the degradation of α- and β-endosulfan in HUIs (Table 2). In conclusion, strain JBW4 had a
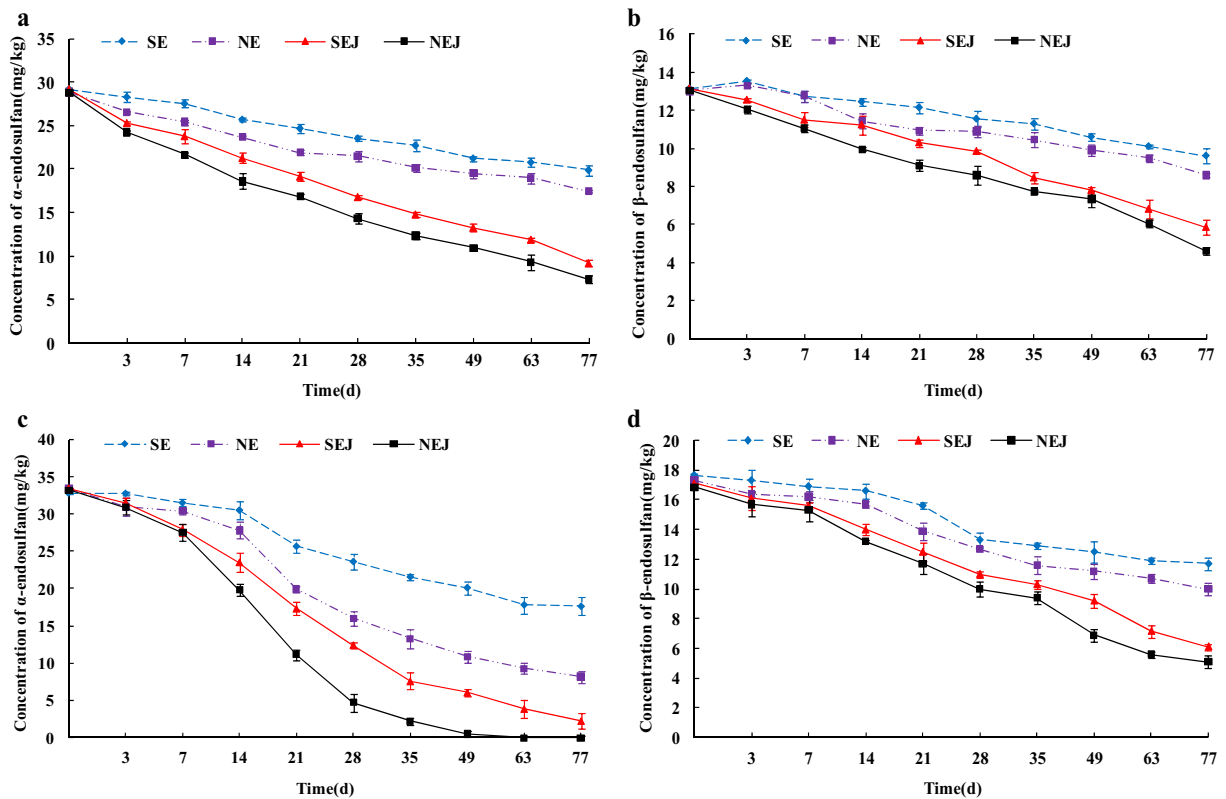
Fig. 1 Time course of α-endosulfan and β-endosulfan degradation in Argi-Udic Ferrosols (**a** and **b**) and Hapli-Udic Isohumosols (**c** and **d**) respectively after different treatments. *SE* sterilized soil with endosulfan, *NE* non-sterilized soil with endosulfan, *SEJ* sterilized soil with endosulfan and JBW4, *NEJ* non-sterilized soil with endosulfan and JBW4. Values presented are the mean ± standard deviation (SD) of three replicates

facilitated impact on the degradation of both endosulfan isomers compared to those uninoculated with JBW4 in AUFs as well as in HUIs. Kong et al. (2013b) reported that strain JBW4 enhanced the degradation of endosulfan in brown soils. These results illustrated that strain JBW4 is an excellent degrader for bioremediation in brown soils, Argi-Udic Ferrosols and Hapli-Udic Isohumosols contaminated with endosulfan.

The degradation rate for α-endosulfan was higher compared with β-endosulfan in AUFs and HUIs, which revealed that strain JBW4 may had more affinity towards α-endosulfan for degradation as suggested by Giri and Rai (2012). They reported that the degradation rate of α-endosulfan is much higher than that of β-endosulfan in both broth culture and soil microcosm. Goswami and Singh (2009) also observed that α-endosulfan was degraded faster than β-endosulfan in the soil after microbial degradation. Beyers et al. (1965) reported that β-endosulfan was more strongly sorbed to the soil than the α-endosulfan, which may be the reason.

The data indicated that the endosulfan in HUIs was degraded faster than in AUFs. Particularly, α-endosulfan was degraded completely on the 63rd day in the NEJ treatment in HUIs (Fig. 1c). In 77 days, treatment NEJ showed a percent degradation of 100, 87, and 74.1% of α-endosulfan in HUIs, brown soil (Kong et al. 2013b), and AUFs. The degradation of β-endosulfan was measured as 69.8, 69.5, and 67.2% in HUIs, brown soil (Kong et al. 2013b), and AUFs. Therefore, after 77 days, the degradation rate of endosulfan in HUIs was the highest among three types of soil (AUFs, HUIs, brown soils). A possible explanation for this could be the most organic matter content in HUIs among three types of soil. The content of organic matter was 23.46, 17.6, and 9.62 g/kg, in HUIs, brown soils, and AUFs, respectively. Strain JBW4 grew best as there was the most easily available nutrient substance in HUIs which was beneficial to the growth and activity of strain JBW4 among three types of soil (Øvreas and Torsvik 1998). Enrichment of the strain JBW4 in HUIs contributed to the

Table 2 Kinetic data for degradation of endosulfan in Argi-Udic Ferrosols and Hapli-Udic Isohumosols with different treatments

Treatments	Argi-Udic Ferrosols						Hapli-Udic Isohumosols					
	C_0 (mg/kg)		Degradation equation	K^a	Half-life (day)[b]	R^2	C_0 (mg/kg)		Degradation equation	K^a	Half-life (day)[b]	R^2
SE	42.28	α	$y = 28.321 \exp(-0.0053x)$	0.0053	129.3	0.9630	50.01	α	$y = 33.173 \exp(-0.0108x)$	0.0108	63.9	0.9651
		β	$y = 13.303 \exp(-0.0039x)$	0.0039	175.0	0.9804		β	$y = 17.548 \exp(-0.0066x)$	0.0066	105.8	0.9204
NE	42.03	α	$y = 26.550 \exp(-0.0062x)$	0.0062	111.9	0.9030	50.19	α	$y = 33.276 \exp(-0.0217x)$	0.0217	31.85	0.9539
		β	$y = 12.886 \exp(-0.0042x)$	0.0042	162.6	0.8461		β	$y = 16.833 \exp(-0.0084x)$	0.0084	82.32	0.9216
SEJ	42.28	α	$y = 26.344 \exp(-0.0164x)$	0.0164	42.1	0.9809	50.81	α	$y = 34.818 \exp(-0.0331x)$	0.0331	20.9	0.9778
		β	$y = 12.826 \exp(-0.0105x)$	0.0105	65.7	0.9902		β	$y = 16.822 \exp(-0.0137x)$	0.0137	50.46	0.9884
NEJ	42.03	α	$y = 24.969 \exp(-0.0214x)$	0.0214	32.4	0.9811	50.06	α	$y = 33.765 \exp(-0.0425x)$	0.0425	16.3	0.9580
		β	$y = 12.131 \exp(-0.0113x)$	0.0113	50.4	0.9760		β	$y = 16.882 \exp(-0.0180x)$	0.0180	38.5	0.9966

SE sterilized soil with endosulfan, *NE* non-sterilized soil with endosulfan, *SEJ* sterilized soil with endosulfan and JBW4, *NEJ* non-sterilized soil with endosulfan and JBW4

[a] K was calculated by using the measured endosulfan residue concentration at different time points in different treatments

[b] The half-life was calculated when $C = 0.5\ C_0$. R correlation coefficient

colonization of JBW4 and lead to the greatest metabolism of endosulfan (Goswami et al. 2009). The degradation rates of endosulfan in AUFs were the slowest among three types of soil. A possible explanation could be the acidic pH value of AUFs. Kong et al. (2013a) reported that the acidic pH value could inhibit the growth of strain JBW4, and strain JBW4 grew best when pH was 7.0 in the study of endosulfan degradation in liquid culture. Therefore, AUFs was too acidic for strain JBW4, which may lead to the least degradation rate of endosulfan among three types of soil.

3.3 Metabolites Formed in the Soil Microcosm

The full-scan raw chromatogram of endosulfan metabolites formed in AUFs and HUIs in NEJ treatment after 77 days are presented in Fig. 2. The metabolites, formed in AUFs, were identified as endosulfan ether and endosulfan lactone, as they co-migrate with the authentic compounds, respectively (Fig. 2a). The metabolites formed in HUIs were the same with metabolites formed in AUFs (Fig. 2b). Additionally, there was no endosulfan sulfate residue in AUFs and HUIs which was reported as the terminal by the indigenous microorganisms in soils (Antonious and Byers 1997; Kataoka and Takagi 2013). Kong et al. (2013b) previously also reported that endosulfan was metabolized to endosulfan ether and endosulfan lactone in JBW4 treatment of brown soils (Kong et al. 2013b). Kamei et al. (2011) found similar findings that the metabolites of endosulfan by white-rot fungus *Trametes hirsute* were endosulfan ether and endosulfan lactone in the culture containing endosulfan diol as a parent substrate. Kullman and Matsumura (1996) proposed that endosulfan was hydrolyzed initially resulting in the intermediate metabolite endosulfan diol and endosulfan diol was converted to other metabolites. Therefore, it was proposed that treatment inoculated with JBW4 generated endosulfan diol as the first step during the degradation of endosulfan, and further converted intermediate product endosulfan diol to endosulfan lactone and endosulfan ether (Kataoka and Takagi 2013; Kong et al. 2013b). It can be inferred that endosulfan was degraded by hydrolysis in AUFs, HUIs, and brown soils (Kong et al. 2013b). Therefore, degradation in treatment inoculated with JBW4 was capable of minimizing the long-term toxicity of endosulfan without the production of endosulfan sulfate in AUFs, HUIs, and brown soils.
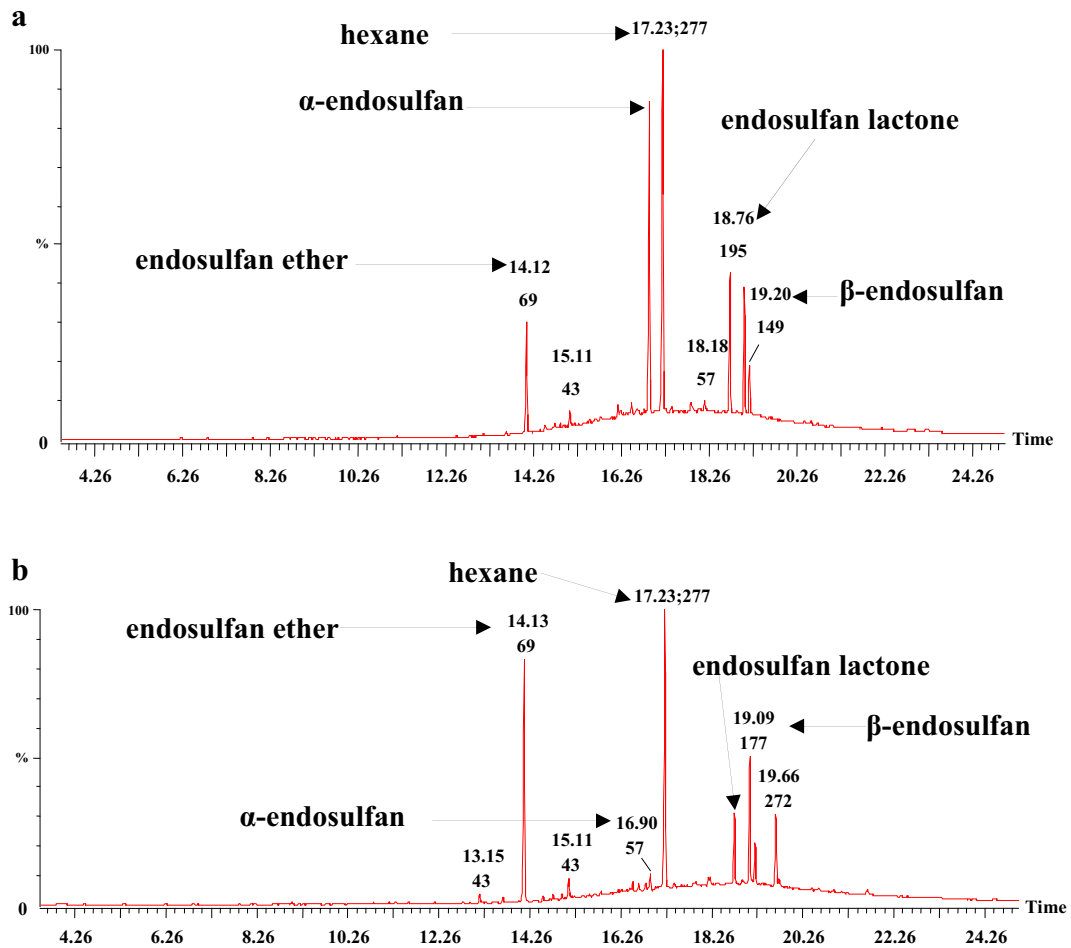
Fig. 2 Mass spectra of endosulfan metabolites in Argi-Udic Ferrosols (**a**) and Hapli-Udic Isohumosols (**b**) of 77 days of group NEJ

3.4 Colonization Analysis of JBW4 from AUFs and HUIs

Using the technique of PCR-DGGE, Altenburger et al. (2010) reported that protozoa and their bacterial prey were able to colonize sterile soil. In this experiment, DGGE was conducted using PCR product from the samples in AUFs and HUIs and the findings are shown in Fig. 3. Zhan et al. (2013) reported that the bright band in the DGGE profile represent the dominant microorganisms in the sample. It was also reported that the brightness of the band represented the richness of the bacteria (Rao et al. 2010). The P band was the target band in Fig. 3a, b. Comparing lanes 3, 5, and 7 with lanes 8 and 9 in Fig. 3b, the brightness of the P band revealed that strain JBW4 colonized non-sterilized soils in HUIs successfully. Similarly, the P appeared

in lanes 2, 4, and 6 (Fig. 3b) and the brightness of P indicated the successful survival and colonization of JBW4 in sterilized soils. This also occurred in AUFs as shown in Fig. 3a. Kong et al. (2013b) reported the similar results that strain JBW4 was able to colonize non-sterilized and sterilized soils using the same technique. These results suggested that strain JBW4 was able to compete with indigenous microbial community and potentially survive and propagate in three types of soil (Castillo et al. 2011; Kong et al. 2013b).

3.5 Toxicity Effect of Endosulfan and its Metabolites in Earthworm Coelomocytes

Figure 4 represents the olive tail moment (OTM) value after 3, 21, 49, and 77 days incubation time in the treatments of AUFs and HUIs. The OTM was used to
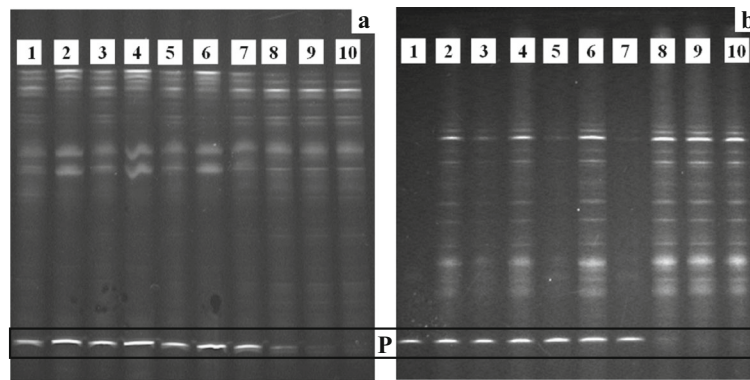
Fig. 3 DGGE profiles of the V3 region amplified by PCR from the DNA extracted from Argi-Udic Ferrosols (**a**) and Hapli-Udic Isohumosols (**b**) of various treatments: P target bands (*1*) JBW4 (*2*) 3 days of group SEJ (*3*) 3 days of group NEJ (*4*) 5 days of group SEJ (*5*) 5 days of group NEJ (*6*) 7 days of group SEJ (*7*) 7 days of group NEJ (*8*) 3 days of group NE (*9*) 5 days of group NE (*10*) CK

evaluate the degree of DNA damage which indicated the toxicity of endosulfan and its metabolites. The increasing OTMs indicated that the degree of DNA damage in the earthworms increased. As shown in Fig. 4a, the toxicity potency of endosulfan and its metabolites in NE and NEJ treatments in AUFs showed a downward trend with time. However, the genotoxicity of the NEJ treatment from 3 to 77 days was considerably lower than that of the NE group. In addition, the distinction in toxicity became larger between the two treatments over time, revealing that toxicity was sharply reduced because of the presence of JBW4. This also occurred in HUIs. Thus, the ability of JBW4 made it a suitable strain for detoxification of endosulfan-contaminated AUFs and HUIs. Kong et al. (2013b) reported similar results that the toxicity of endosulfan and its metabolites was reduced rapidly during the biodegradation of endosulfan by JBW4 in brown soils.

The decrease in toxicity of endosulfan in earthworm cells may be attributed to the degradation of endosulfan and formation of less toxic metabolites (Dorough et al. 1978; Sutherland et al. 2002; Romero-Aguilar et al. 2014). Endosulfan ether and lactone produced in AUFs and HUIs were less toxic to mice than endosulfan (Dorough et al. 1978).

Particularly, in Fig. 4b, the OTM values in the NEJ treatment were close to those in the control at day 77 in HUIs, which suggested that the toxicity resulted from endosulfan residue in the NEJ treatment after degradation was slight in earthworm coelomocytes. Romero-Aguilar et al. (2014) reported similar findings that the toxicity of endosulfan was decreased 100% through comet assay after *Penicillium* sp. degradation. Taken together, these results indicated that the addition of JBW4 could not only accelerate the degradation of endosulfan but also mitigate the
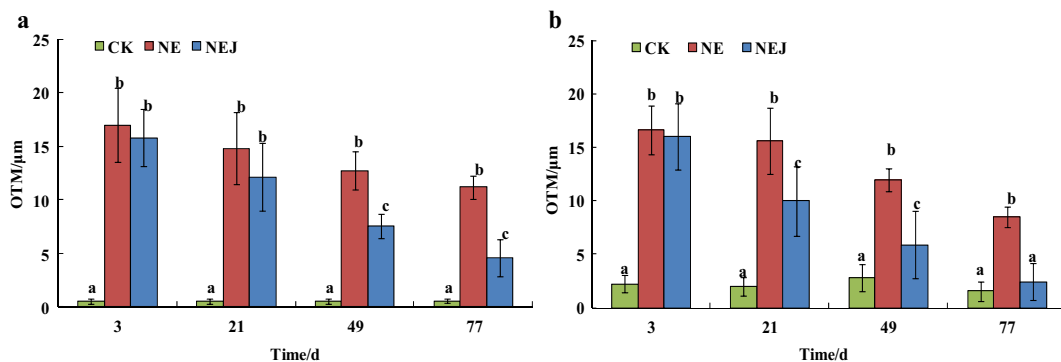

Fig. 4 Effect of endosulfan exposure on OTM in earthworm (*Eisenia fetida*) cells in Argi-Udic Ferrosols (**a**) and Hapli-Udic Isohumosols (**b**) at 3, 21, 49, and 77 days (*error bars* represent mean ± SD, $n = 3$). The *lowercase letters* above the histograms indicate significant differences among treatments ($p < 0.05$). *CK* non-sterilized soil, *NE* non-sterilized soil with endosulfan, *NEJ* non-sterilized soil with endosulfan and JBW4

toxicity of endosulfan-contaminated soil. The toxicity potency of endosulfan and its metabolites in three types of soil after degradation ranked as follows: AUFs > brown soil > HUIs. The order was in agreement with that of the degradation rates in three types of soil as the degradation rates of endosulfan isomers in HUIs were the highest.

4 Conclusion

Based on the present studies, we can conclude that bacterial strain JBW4 has a potential to be applied for bioremediation of endosulfan-contaminated soils. The major degradation metabolites were endosulfan lactone and endosulfan ether in AUFs and HUIs. Strain JBW4 colonized AUFs and HUIs and improved the degradation rates of endosulfan in AUFs and HUIs compared with those in the uninoculated treatment significantly. The degradation rates of endosulfan were higher and the toxicity was smaller in HUIs compared with those in AUFs. The toxicity of endosulfan and its metabolites in the two soils decreased significantly after microbial degradation by strain JBW4.

Compliance with Ethical Standards

Conflict of Interest   The authors declare that they have no competing interests.

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