J. Microbiol. Biotechnol. (2017), 27(7), 1306–1315
https://doi.org/10.4014/jmb.1701.01073

jmb

Evaluation of the Synergistic Effect of Mixed Cultures of White-Rot Fungus *Pleurotus ostreatus* and Biosurfactant-Producing Bacteria on DDT Biodegradation

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Received: January 31, 2017
Revised: March 21, 2017
Accepted: April 20, 2017

First published online
April 21, 2017

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pISSN 1017-7825, eISSN 1738-8872

DDT (1,1,1-trichloro-2,2-*bis*(4-chlorophenyl) ethane) is one of the organic synthetic pesticides that has many negative effects for human health and the environment. The purpose of this study was to investigate the synergistic effect of mixed cutures of white-rot fungus, *Pleurotus ostreatus*, and biosurfactant-producing bacteria, *Pseudomonas aeruginosa* and *Bacillus subtilis*, on DDT biodegradation. Bacteria were added into the *P. ostreatus* culture (mycelial wet weight on average by 8.53 g) in concentrations of 1, 3, 5, and 10 ml (1 ml ≈ 1.25×10^9 bacteria cells/ml culture). DDT was degraded to approximately 19% by *P. ostreatus* during the 7-day incubation period. The principal result of this study was that the addition of 3 ml of *P. aeruginosa* into *P. ostreatus* culture gave the highest DDT degradation rate (approximately 86%) during the 7-day incubation period. This mixed culture combination of the fungus and bacteria also gave the best ratio of optimization of 1.91. DDD (1,1-dichloro-2,2-*bis*(4-chlorophenyl) ethane), DDE (1,1-dichloro-2,2-*bis*(4-chlorophenyl) ethylene), and DDMU (1-chloro-2,2-*bis*(4-chlorophenyl) ethylene) were detected as metabolic products from the DDT degradation by *P. ostreatus* and *P. aeruginosa*. The results of this study indicate that *P. aeruginosa* has a synergistic relationship with *P. ostreatus* and can be used to optimize the degradation of DDT by *P. ostreatus*.

Keywords: Biodegradation, DDT, *Pleurotus ostreatus, Pseudomonas aeruginosa, Bacillus subtilis*

Introduction

Environmental pollution is an inseparable part of human life. Increasing human needs have encouraged the growth of industries, leading to the production of new products and the use of modern technologies. Among the various kinds of environmental pollutants, synthetic pesticides from the agricultural industry now constitute a serious environmental problem. Organochlorine pesticides have been widely used in the fields of agriculture and health since the 1940s. DDT (1,1,1-trichloro-2,2-*bis*(4-chlorophenyl) ethane) was introduced as an organochlorine pesticide during World War II to combat mosquitoes that spread malaria and typhus [1–3]. DDT was the first synthetic pesticide to gain popularity all over the world and it is still being used in some developing countries for essential public health and agricultural purposes [3]. However, DDT has been prohibited in most countries owing to its negative impacts on wildlife and its ill effects on human health [3, 4].

Although DDT has been banned, its residues are still present in the environment in high concentrations, especially in flowing water bodies and soil around agricultural areas [1, 5, 6]. Some treatment methods that work by degrading the compound have been developed to reduce and minimize the adverse impacts of the use of DDT. Among the several methods used, biodegradation is the most secure, efficient, and relatively low-cost method. Biodegradation uses microorganisms (fungi and bacteria) as agents to degrade DDT, by converting the compound into a form that is either less toxic or not toxic at all.

The ability of microorganisms, particularly white-rot fungi, to degrade DDT has been investigated by researchers. White-rot fungi have ligninolytic enzyme systems that play a role in degrading DDT. An example of such white-rot fungi is *Pleurotus ostreatus*. Purnomo *et al*. [7] reported that *P. ostreatus* was capable of degrading DDT to approximately 45% in potato dextrose broth (PDB) medium during a 14-day incubation. In addition, spent mushroom waste (SMW) of *P. ostreatus* degraded and mineralized DDT to approximately 48% and 51% during 28-day and 56-day incubation periods, respectively [8]. The SMW *P. ostreatus* was also applied to degrade DDT in sterilized and un-sterilized soils to 40% and 80%, respectively, in a 28-day incubation period. These reports indicate that *P. ostreatus* is a potential fungus for the biodegradation of DDT in pure culture as well as in contaminated soil. However, the amount of DDT degradation by *P. ostreatus* is still low and requires a long incubation time. Therefore, it is necessary to modify the culture so as to enhance its ability in degrading DDT.

This study examined the optimization of DDT degradation by *P. ostreatus* through the addition of biosurfactant-producing bacteria, *Pseudomonas aeruginosa* [9–15] and *Bacillus subtilis* [16–18]. Since DDT is a hydrophobic compound, the addition of biosurfactant can increase the solubility of DDT thereby optimizing the process. Apart from being able to produce biosurfactant, *P. aeruginosa* is also capable of degrading the compounds of pollutants, including DDT [19, 20]. *P. aeruginosa* can degrade DDT to several metabolic products such as DDMU (1-chloro-2,2-*bis*(4-chlorophenyl) ethylene), DDMS (1-chloro-2,2 *bis* (4′-chlorophenyl) ethane), DDOH (2,2-*bis* (*p*-chlorophenyl) ethanol), DDA (*bis* (*p*-chlorophenyl) acetic acid), 2-chlorobiphenyl, 4-chlorophenyl, 4-chlorobenzoic acid, and also two polar metabolic products, catechol and hydroquinone [19]. In addition, *P. aeruginosa* strain 640X degrades DDT to non-chlorinated metabolic products such as phenylacetate, phenylpropnioate, and salicylic acid [20]. *B. subtilis*, on the other hand, can degrade organic pollutants such as dyes [21, 22], aflatoxin [23], diethyl phthalate [24], and DDT [25, 26]. Given these properties, the effect of the addition of biosurfactant-producing bacteria on DDT degradation by *P. ostreatus* was investigated and the metabolic products were identified.

Materials and Methods

Chemicals

DDT was purchased from Tokyo Chemical Industry Co. (Japan). Acetone, methanol, *n*-hexane, and *N,N*-dimethylformamide (DMF) were purchased from Wako Pure Chemical Industries (Japan).

Fungus Culture Conditions

Stock cultures of *P. ostreatus* BM9073 were maintained on 9-cm-diameter potato dextrose agar plates (PDA; Difco, USA) that had been incubated at 25°C. The mycelia from the agar plate were transferred to a sterile blender cup containing 25 ml of sterile water and then homogenized for 30 sec. One milliliter of this homogenate was inoculated into 10 ml of PDB (Difco) medium in a 100-ml Erlenmeyer flask. The cultures were pre-incubated statically at 25°C for 7 days [27–29].

Bacteria Culture Conditions

Stock cultures of *P. aeruginosa* NBRC 3080 and *B. subtilis* NBRC 3009 were maintained on 9-cm-diameter nutrient agar (NA; Difco) that had been incubated at 37°C. The colony was inoculated into 40 ml of nutrient broth (NB; Difco) medium in 50-ml Falcon flasks. The cultures were pre-incubated at 37°C for 12 h with a shaker (180 rpm) [30].

Biodegradation of DDT by *P. ostreatus*

After pre-incubation for 7 days, 50 µl of 5 mM DDT in DMF was added to each flask inoculated with *P. ostreatus* (final concentration, 0.25 µmol). The headspace of each flask was flushed with oxygen and then sealed with a glass stopper and sealing tape, to prevent the volatilization of substrate. The cultures were incubated statically for 7 days at 25°C. As a control, the cultures were killed by autoclaving (121°C, 20 min) after pre-incubation. The experiments were performed in triplicates [7, 27–29].

Biodegradation of DDT by Biosurfactant Producing Bacteria

After pre-incubation for 12 h, bacteria cultures were inoculated separately into PDB culture (final volume 10 ml) at different concentrations of 1, 3, 5, and 10 ml (1 ml $\approx$ 1.25×10^9 bacteria cells/ml culture). Fifty microliters of 5 mM DDT in DMF (final concentration, 0.25 µmol) was added to each bacteria-inoculated flask. The headspace of each flask was flushed with oxygen and then sealed with a glass stopper and sealing tape to prevent the volatilization of substrate. The cultures were incubated statically for 7 days at 25°C. As a control, the cultures were killed by autoclaving (121°C, 20 min) after pre-incubation. The experiments were performed in triplicates.

Biodegradation of DDT by *P. ostreatus* with Addition of Biosufactant-Producing Bacteria

After fungus pre-incubation for 7 days, bacteria cultures were added separately into the *P. ostreatus* culture at concentrations of 1, 3, 5, and 10 ml (1 ml $\approx$ 1.25×10^9 bacteria cells/ml culture). Fifty microliters of 5 mM DDT in DMF (final concentration, 0.25 µmol) was added to each flask containing the mixed culture. To prevent the volatilization of substrate, the flask was sealed with a glass stopper and sealing tape after the headspace of each flask had been flushed with oxygen. The cultures were incubated statically

for 7 days at 25°C. As a control, the cultures were terminated by autoclaving (121°C, 20 min) after pre-incubation. The experiments were performed in triplicates. The ratio optimization (RO) was calculated as amount of degradation by mixed cultures per total amounts of degradation by fungus and bacteria.

Analytical Method

Fifty microliters of 5 mM pyrene in DMF (final concentration, 0.25 μmol) was added to each of the samples as an internal standard. The mixtures were homogenized with 40 ml of methanol and then washed with 120 ml of acetone. After filtration through a glass fiber filter (1.0 μm pore size; Advantec, Japan), the filtrates were mixed and evaporated. The filtrates were extracted with 250 ml of *n*-hexane and the organic fraction was collected and dried over anhydrous sodium sulfate. The extracts were evaporated and concentrated to dryness under reduced pressure. The concentrate was diluted with methanol and then analyzed by high-performance liquid chromatography (HPLC; Jasco, Japan) to quantify the amount of DDT. The HPLC system consisted of a PU-1500 intelligent pump (Jasco) and an MD-1510 multiwavelength detector (Jasco) fitted with an Inertsil ODS-3 column (150 mm) with an inner diameter of 4.6 mm (GL Science, Japan).

The samples were eluted with 82% methanol in a 0.1% trifluoroacetic acid aqueous solution at a flow rate of 1 ml/min. DDT and its metabolic products were identified on the basis of the retention time and absorption maximum at specific wavelengths in comparison with authentic standards. For quantitative analysis, the peak areas of DDT and its metabolites were compared with the peak area of pyrene [29]. To identify metabolic products that could not be detected by HPLC, samples were further diluted with *n*-hexane and then analyzed by gas chromatography/mass spectrometry (GC/MS; HP, USA). GC/MS was performed on an HP 6890 GC system (HP) linked to an HP 5973 mass-selective detector (HP) with a 30-m fused DB-5MS column (J&W Scientific, USA). The oven temperature was held at 80°C for 3 min, followed by a linear increase to 300°C at 20°C/min and it was held at this temperature for 5 min. The injector temperature was set at 250°C. Injection was splintless at approximately 1 μl [27, 31, 32].

Statistical Analysis

The results were calculated as the average of triplicate determinations. The Student's *t*-test was used to detect any significant differences between or within groups during DDT transformation. Differences between means at a confidence level of 5% ($p < 0.05$) were considered to be statistically significant [33, 34].

Results and Discussion

Many fungi have been reported to have abilities for metabolizing DDT, such as *Aspergillus flavus*, *Aspergillus niger*, *Penicillium brefeldianum* [35], *Pleurotus sajor-caju*, *Pleurotus florida*, *Pleurotus eryngii* [36], *P. chrysosporium* [36–38], *Pleurotus ostreatus* [7, 8, 31, 37], *Phlebia lindtneri*, and *Phlebia brevispora* [39], as well as brown-rot fungi *Gloeophyllum trabeum*, *Daedalea dickinsii*, and *Fomitopsis pinicola* [7, 27, 29, 31]. It has been reported in previous studies that *P. ostreatus* is capable of degrading DDT in pure liquid medium [7], SMW, as well as in contaminated soil [8]. However, the degradation amount is still low and the process requires a long incubation time. Therefore, in this study, the ability of *P. ostreatus* to degrade DDT was enhanced through the addition of biosurfactant-producing bacteria. *P. aeruginosa* and *B. subtilis* have been reported as biosurfactant-producing bacteria [9, 11, 13–18], which can increase the solubility of hydrophobic organic substances, including DDT, to optimize the access of microbial uptake. In this study, degradation occurred for 7 days due to a shorter incubation time than that reported in previous studies (14 d) [7] as well as to compare the effectiveness of addition of bacteria on DDT degradation by *P. ostreatus*.

Degradation of DDT by Individual Organisms

DDT degradation by the individual fungus or bacterium is shown in Table 1. *P. ostreatus* only degraded DDT at an amount of approximately 19% during the 7-day incubation time. In the case of DDT degradation by bacteria, it was observed that the higher the concentration of *P. aeruginosa*, the higher was the degradation rate obtained. *P. aeruginosa* started to degrade DDT at concentrations from 1 to 5 ml, and the highest degradation rate of approximately 36% was recorded at the 5 ml concentration. However, addition of excess *P. aeruginosa* (10 ml) resulted in a decrease in DDT degradation amount (26%) due to competition for survival among the bacteria rather than promotion of DDT degradation. In an abundant population of bacteria in the stationery phase, the bacteria produce some secondary metabolites, which may be toxic for others in order to survive. On the other hand, *B. subtilis* was also capable of degrading DDT, and the higher the concentration of *B. subtilis*, the higher was the degradation of DDT obtained (Table 1). At a concentration of 1 ml, *B. subtilis* could not degrade DDT owing to the low number of bacteria. However, *B. subtilis* started to degrade DDT at a concentration of 3 ml, and the highest DDT degradation by *B. subtilis* was obtained at a concentration of 10 ml with a degradation amount of approximately 42%. Based on these results, *B. subtilis* had a higher degradation amount than *P. aeruginosa* in PDB medium during the 7-day incubation period.

Many bacteria have been reported to have capabilities to degrade DDT, such as *Aerobacter aerogenes* [40, 41], *Trichoderma viridae*, *Micrococcus* sp., *Arthrobacter* sp., *Bacillus* sp. [42],

Table 1. Degradation of DDT by individual cultures of *P. ostreatus* or bacteria in PDB medium during a 7-day incubation period.

Microorganims	Bacteria concentration (ml)	Recovery (%)		Degradation amount (%)
		Control	Treatment	
P. ostreatus	0	98.56 ± 4.78*	79.22 ± 1.85*	19.34
P. aeruginosa	1	99.08 ± 2.80	95.96 ± 2.26	3.12
	3	102.91 ± 4.21*	77.36 ±5.92*	25.55
	5	103.15 ± 4.60*	67.08 ± 3.01*	36.07
	10	97.78 ± 1.36*	71.55 ± 6.17*	26.23
B. subtilis	1	98.49 ± 6.90	98.49 ±3.43	0.00
	3	97.79 ± 3.93*	77.72 ± 2.63*	20.07
	5	95.49 ± 2.87*	67.63 ± 3.15*	27.86
	10	97.75 ± 0.72*	56.1 ± 4.79*	41.65

Analyses were conducted using HPLC. Data are the mean ± standard deviation (n = 3). Within each row, values followed by (*) are significantly different ($p < 0.05$). One milliliter of bacteria concentration is equal to 1.25×10^9 bacteria cell/ml culture. PDB, potato dextrose broth.

Hydrogenomonas sp. [43–45], *Alcaligenes eutrophus* A5 [46], *Pseudomonas putida* [35], *Bosea thiooxidans* [47], *Pseudomonas* sp. [42, 48], and *Sphingobacterium* sp. [49]. Bidlan [19] reported that *P. aeruginosa* strain DT-Ct1 degraded 5 ppm DDT completely in 96 h. Langlois *et al*. [26] had reported that whole cells of *Bacillus cereus*, *Bacillus coagulans*, *B. subtilis*, *Escherichia coli*, and *Enterobacter aerogenes* degraded DDT in trypticase soy broth medium. *Bacillus* sp. degraded DDT by 51% in liquid culture during a 7-day incubation period [50]. It has also been reported that *P. aeruginosa* and *B. subtilis* are capable of degrading some persistent organic pollutants, including DDT [19, 20, 25, 26]. The bacterium *Rhodococcus* sp. ITR03 was reported to produce biosurfactants (trehalolipid and rhamnolipid) as well as degrade DDT residues and chlorinated aromatic compounds [51]. Thus, the results indicate that both bacteria, *P. aeruginosa* and *B. subtilis*, had the ability to degrade DDT in addition to producing biosurfactants. Therefore, they might be potential bacteria for enhancing DDT degradation by the fungus *P. ostreatus*.

Degradation of DDT by Mixed Cultures

The analysis results of biodegradation of DDT by *P. ostreatus* with the addition of bacteria are shown in Table 2. From HPLC analysis, the amounts of degradation of DDT by *P. ostreatus* with the addition of *P. aeuginosa* at concentrations of 1, 3, 5, and 10 ml were approximately 21%, 86%, 69%, and 30%, respectively. In addition, the amounts of DDT degradation for *P. ostreatus* on the addition of *B. subtilis* at concentrations of 1, 3, 5, and 10 ml were approximately 25%, 30%, 43%, and 9%, respectively. These results indicate that the addition of bacteria resulted in increasing DDT degradation rates by *P. ostreatus*. Degradation of organic pollutants by fungal-bacterial mixed cultures

Table 2. Degradation of DDT by mixed cultures of *P. ostreatus* and bacteria in PDB medium during a 7-day incubation period.

Microorganims	Bacteria concentration (ml)*	Recovery (%)**		Degradation amount (%)	Ratio optimization
		Control	Treatment		
P. ostreatus	0	98.56 ± 4.78*	79.22 ± 1.85*	19.34	-
Mix *P. osteatus & P. aeruginosa*	1	99.08 ± 2.80*	78.28 ± 7.51*	20.80	0.93
	3	102.91 ± 4.21*	17.17 ± 3.05*	85.74	1.91
	5	103.15 ± 4.60*	34.20 ± 4.47*	68.95	1.24
	10	97.78 ± 1.36*	68.26 ± 1.94*	29.52	0.65
Mix *P. ostreatus & B. subtilis*	1	98.49 ± 6.90*	73.68 ± 4.60*	24.81	1.28
	3	97.79 ± 3.93*	67.45 ± 0.72*	30.34	0.77
	5	95.49 ± 2.87*	52.49 ± 6.46*	43.00	0.91
	10	97.75 ± 0.72*	89.29 ± 2.79*	8.46	0.14

Analyses were conducted using HPLC. Data are the mean ± standard deviation (n = 3). Within each row, values followed by (*) are significantly different ($p < 0.05$). One milliliter of bacteria concentration is equal to 1.25×10^9 bacteria cell/ml culture. PDB, potato dextrose broth.

has been previously reported. Wang *et al*. [52] reported that the fungal-bacterial mixed cultures removed the highest amounts of pyrene (67%) during a 28-day incubation, compared with mixed fungal cultures (39%) and mixed bacterial cultures (56%), as well as in the degradation of phenanthrene and fluoranthene. Hai *et al*. [53] reported pesticide removal by a mixed culture of bacteria from sludge and white-rot fungus *Coriolus versicolor*, in which the concentrations of aldicarb, atrazine, and alachlor dropped by 82%, 77%, and 67%, respectively, after incubation for 7 days. Furthermore, the addition of an exogenous aerobic bacteria mixture (*Bacillus cereus*, *B. subtilis*, *Staphylococcus saprophyticus*, *Staphylococcus xylosus*, and *Pantoea agglomerans*) on white-rot fungus *Phanerochaete chrysosporium* effectively enhanced the hydrolysis and acidogenesis processes of municipal solid waste degradation [54].

Fig. 1 compares the individual DDT degradation rates of the fungus *P. ostreatus* and the bacterium *P. aeruginosa* with those of their mixed cultures. The addition of *P. aeruginosa* into *P. ostreatus* culture had an influence on the degradation of DDT. Higher DDT degradation rates were obtained on addition of *P. aeruginosa* than for *P. ostreatus* only. The addition of 3 ml of *P. aeruginosa* in cultures of *P. ostreatus* produced the highest degradation of about 86%. Synergism between the fungus and the bacteria might be caused by the ability of *P. aeruginosa* to produce a rhamnolipid biosurfactant form, which can improve the solubility of DDT for degradation [10, 55]. In addition to producing biosurfactant, this bacterium is also capable of metabolizing DDT [19, 20]. However, the addition of excess *P. aeruginosa* resulted in a decrease in DDT degradation by *P. ostreatus* due to increased competition for nutrients and the production of some toxic metabolites for survival rather than the degradation of DDT. Rhamnolipid biosurfactants, which are produced by *P. aeruginosa*, also have antifungal activity [56, 57], and this might inhibit *P. ostreatus* from degrading DDT.

Fig. 2 compares the individual DDT degradation rates of the fungus *P. ostreatus* and the bacterium, *B. subtilis* with those of their mixed cultures. The addition of *B. subtilis* in the culture of *P. ostreatus* had a varying effect on the process of DDT degradation. The degradation amounts increased on the addition of 1, 3, and 5 ml of *B. subtilis*, but decreased on addition of 10 ml of *B. subtilis*. The addition of 5 ml of *B. subtilis* in cultured *P. ostreatus* produced the highest degradation rate of about 43%. *B. subtilis* has been reported to produce lipopeptide biosurfactants, which improve solubility of non-polar substances [17, 58]. In addition, the bacteria are able to degrade DDT [25, 26]. However, as was observed with *P. aeruginosa*, the addition of excess *B. subtilis* (10 ml) resulted in an antagonistic relationship, thereby decreasing the DDT degradation amount. *B. subtilis* also produces biosurfactants that have antifungal activity [57], and this might inhibit *P. ostreatus* from degrading DDT. Furthermore, *B. subtilis* is known to produce secondary metabolites, including cyclic lipopeptides, polypeptides, proteins (enzymes), and non-peptide products, which have antimicrobial properties [59]. Enhancement of

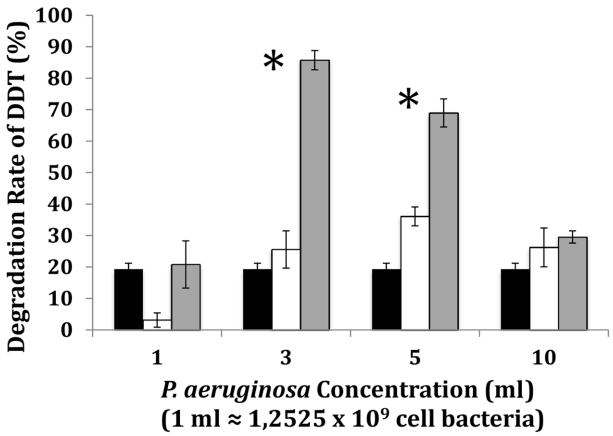

Fig. 1. DDT degradation amounts for white-rot fungus (*P. ostreatus*) only (black bars), bacteria (*P. aeruginosa*) only (white bars), and mixed cultures of *P. ostreatus* and *P. aeruginosa* (grey bars) during incubation for 7 days.
Data points are the means and standard deviations (n = 3). The (*) on each bar indicates significant differences ($p < 0.05$).

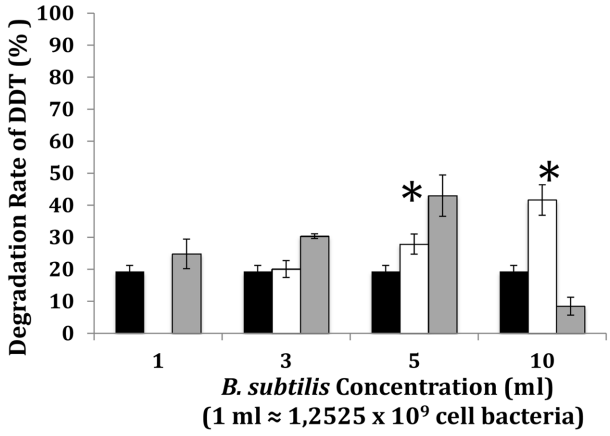

Fig. 2. DDT degradation amounts for white-rot fungus (*P. ostreatus*) only (black bars), bacteria (*B. subtilis*) only (white bars), and mixed cultures of *P. ostreatus* and *B. subtilis* (grey bars) during the 7-day incubation period.
Data points are the means and standard deviations (n = 3). The (*) on each bar indicates significant differences ($p < 0.05$).

Table 3. Enhancement of biodegradation of DDT by microorganisms with additional sources.

Species	Degradation amount	Incubation time	Metabolites products	Addition sources	References
Serratia marcescens	100%	72 Hours	Not reported	Yeast extract, peptone, glycerol, tryptone soya broth	[74]
Shewanella decolorationis S12	60%	9 Months	DDD, DDMS, DBP	Iron oxide (α-FeOOH)	[75]
Sphingobacterium sp.	0.031 $mg \cdot l^{-1} \cdot day^{-1}$	21 Days	DDD, DDE, DDMU, DDNS, DDA, DBP	Glucose	[49]
Phanerochaete chrysosporium ACCC 3055	74%	28 Days	DDE, DDD, DBP	Microemulsion: Tween 80, 1-pentanol, linseed oil	[60]
Clostridium sp. BXM	74%	6 Hours	DDD, DDE	Na_2SO_4	[76]
Bacillus thuringiensis	79%	8 Weeks	DDD, DDE	KH_2PO_4, Urea, Tween 80	[61]
Flavobacterium sp.					
Variovorax soli					
Lysobacter sp.					
Bosea sp.					
Bacillus thuringiensis	94%	8 Weeks	DDD, DDE	KH_2PO_4, Urea	[61]
Flavobacterium sp.					
Phenylobacterium sp.					

biodegradation of DDT by a specific bacterium or fungus using additional sources has been reported by various researchers and is shown in Table 3. Tween 80 has been used to enhance DDT degradation by some fungi [60, 61], which indicates that surfactants can improve the DDT degradation amount. However, synthetic surfactants are expensive to use; therefore, the use of biosurfactant-producing bacteria for optimizing fungal cultures is a promising method for application in the bioremediation of DDT.

The degradation abilities of the individual fungus or bacterium were different from those of their mixed cultures. *P. aeruginosa* only showed the highest DDT degradation rate at a concentration of 5 ml, but in the mixed culture, the highest DDT degradation rate was observed when the concentration of *P. aeruginosa* was 3 ml (Fig. 1). This indicates that the concentration of the bacterium affected the synergistic relationship between *P. ostreatus* and *P. aeruginosa*. A similar pattern was also observed with *B. subtilis*. The highest DDT degradation amount was observed at a concentration of 10 ml for *B. subtilis* only; however, the highest DDT degradation amount in the mixed cultures was observed on addition of 5 ml of *B. subtilis* to the *P. ostreatus* culture. Excess concentrations of bacteria might produce more biosurfactants, but this is only beneficial in degradation processes involving only the bacteria. Excess production of biosurfactants might be harmful for fungus, because biosurfactants exhibit antifungal activity.

Ratio Optimization

The effectiveness of compositions of mixed cultures was determined by RO. The RO indicated the level of enhancement of DDT degradation due to the synergistic relationship between *P. ostraeus* and the bacteria, compared with the degradation by the individual organisms. In the case of *P. aeruginosa*, the addition of 3 and 5 ml of the bacterium into *P. ostreatus* culture showed RO > 1, which indicates that DDT degradation by the mixed cultures was higher than the total of those of the individual fungus and bacterium (Table 2, Fig. 1). The addition of 3 ml of *P. aeruginosa* showed the highest RO, which enhanced the degradation by approximately 2 folds. The addition of 10 ml of *P. aeruginosa* showed the lowest RO, although the degradation rate was higher than that of 1 ml of *P. aeruginosa*. On the other hand, addition of *B. subtilis* in mixed cultures showed RO < 1, except in the addition of 1 ml of *B. subtilis* where no DDT degradation was observed for 1 ml of *B. subtilis* only. This indicates that, even though 1 ml of *B. subtilis* only could not degrade DDT, 1 ml of *B. subtilis* in the mixed culture could produce enough biosurfactants to enhance the degradation process. Based on these results, *P. ostreatus* and 3 ml of *P. aeruginosa* gave the best-mixed culture combination with a RO of 1.91 (Table 2), as well as the highest degradation of DDT. Thus, it was selected for further experiments on identification of metabolic products.

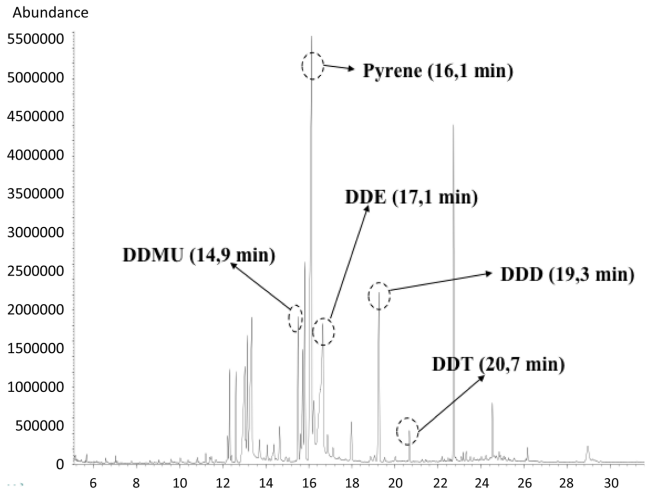

Fig. 3. GC/MS chromatogram of metabolite products of DDT degradation by mixed cultures of *P. ostreatus* and *P. aeruginosa*.

Identification of Metabolic Products

The result of GC MS analysis showed that the mixed culture of *P. ostreatus* and *P. aeruginosa* (3 ml) was able to degrade DDT to some metabolites, including DDE, DDD, and DDMU (Fig. 3). These metabolites were the same as those produced by *P. ostreatus* only (data not shown). However, *P. aeruginosa* only transformed DDT to DDE as the end product, which indicates that DDE was subjected to further transformation by *P. ostreatus*.

In general, the bacteria can degrade DDT aerobically or anaerobically. The bacteria are incubated aerobically to degrade DDT via the dehydrochlorination reaction and to give DDE as the metabolic product, whereas DDT is predominantly converted to DDD metabolites anaerobically [5]. Bidlan [19] reported that *P. aeruginosa* degraded DDT to several metabolic products, such as DDMU, DDMS, DDOH, DDA, 2-chlorobiphenyl, 4-chlorophenyl, 4-chlorobenzoic acid, and also two polar metabolic products, catechol and hydroquinone, anaerobically [19]. In addition, *P. aeruginosa* strain 640X degraded DDT to non-chlorinated metabolic products such as phenylacetate, phenylpropnioate, and salicylic acid [20]. However, in this study, the experiment was performed aerobically by adding oxygen, and DDE was detected as the end metabolic product of DDT degradation by *P. aeruginosa*.

In previous reports, spent mushroom of white-rot fungus *P. ostreatus* converted DDT to other metabolites, including DDD, DDMU, DDA, and DDMS as well as mineralization to CO_2 [8]. In the present study, the mixed cultures of *P. ostreatus* and *P. aeruginosa* converted DDT to DDE, DDD, and DDMU. The presence of DDE was due to the conversion by *P. aeruginosa*, whereas DDD and DDMU are attributable to *P. ostreatus*. Moreover, DDE, as end metabolites from *P. aeruginosa*, might be converted by *P. ostreatus* to other metabolites such as DDD and DDMU. However, since DDD and DDMU may not be end products of DDT biotransformation by mixed cultures of *P. ostreatus* and *P. aeruginosa*, further investigation is needed to use DDT metabolites (DDE, DDD, and DDMU) as substrates for degradation by mixed cultures of *P. ostreatus* and *P. aeruginosa*.

In aerobic condition, oxygenase and peroxidase are the key enzymes of aerobic biodegradation. *P. ostreatus* produces oxidative ligninolytic enzymes such as laccase, MnP, and LiP, which are responsible for degrading lignin and pollutants [62]. Among the ligninolytic enzymes, MnP shows high activity in *P. ostreatus* culture [8]. However, *P. ostreatus* was found to metabolize some aromatic compounds by hydroxylation that does not depend on ligninolytic enzymes [63–65]. Intracellular enzymes may also be involved in DDT degradation, and one of them is cytochrome P450 monooxygenase (P450). The P450 enzyme catalyzes a wide variety of reactions such as hydroxylation, oxidation, reduction, dehalogenation, and dehydrogenation, which are involved in the degradation of some pollutants [64–70]. Under aerobic condition, DDT is metabolized to DDD, dicofol, and 2,2-*bis*(4-chlorophenyl) acetic acid (DDA) by P450 [71, 72], and DDT monohydroxylation of aromatic ring by P450 has been proposed as a new metabolic pathway of DDT biodegradation [73]. It is assumed that alongside ligninolytic enzymes, the intracellular enzyme P450 may have an important role in the metabolism of DDT.

This study highlighted the optimization of DDT degradation by white-rot fungus *P. ostreatus* through the addition of biosurfactant-producing bacteria. The addition of 3 ml of *P. aeruginosa* into *P. ostreatus* culture resulted in the highest DDT degradation of approximately 86% in PDB medium during a 7-day incubation period. This fungus-bacterium culture combination also gave the highest optimization ratio of 1.91. DDE, DDD, and DDMU were detected as metabolic products from DDT degradation by mixed cultures of *P. ostreatus* and *P. aeruginosa*. This study demonstrates the effectiveness of addition of bacteria, especially *P. aeruginosa*, on DDT degradation by *P. ostreatus*.

Acknowledgments

This work was supported by a grant from the research project No: 010/SP2H/LT/DRPM/IV/2017 from the Directorate of Research and Community Service, Directorate

General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education, Indonesia.

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