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Isolation and identification of dieldrin-degrading *Pseudonocardia* sp. strain KSF27 using a soil–charcoal perfusion method with aldrin *trans*-diol as a structural analog of dieldrin

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ABSTRACT

We isolated a novel aerobic dieldrin-degrading bacterium from an enrichment culture in a soil–charcoal perfusion system. Enrichment culture using a soil–charcoal perfusion system was an effective way to obtain microorganisms that degrade recalcitrant compounds. The soil–charcoal perfusion was performed using aldrin *trans*-diol, which was a metabolite of dieldrin. Aldrin *trans*-diol had higher bioavailability (2.5 mg/l) than dieldrin (0.1–0.25 mg/l), therefore it is possible for microorganisms to utilize it as a substrate in soil. After 100 days of circulation and three exchanges of the medium, the enriched charcoal was harvested and a bacterium isolated. The isolate was designated as strain KSF27 and was found to be closely related to *Pseudonocardia* spp. as determined by 16S rRNA sequencing analysis. Strain KSF27 degraded aldrin *trans*-diol by 0.05 μmol/l from an initial concentration of 25.5 μmol/l. The metabolite of aldrin *trans*-diol was detected by HPLC/MS and determined to be aldrindicarboxylic acid based on retention time and the MS fragment. Moreover, strain KSF27 degraded dieldrin from 14.06 μmol/l to 2.01 μmol/l over a 10-day incubation at 30 °C. This strain degraded dieldrin and other persistent organochlorine pesticides, such as α-endosulfan, β-endosulfan, endosulfan sulfate, heptachlor, heptachlor epoxide and chlordecone.

1. Introduction

The organochlorine insecticides, including dieldrin, have been extensively used for pest control in agriculture. Although they are very efficient insecticides, their use has been prohibited in many countries since the 1970s because of its biological magnification, high toxicity and long persistence in the environment. Dieldrin in particular was classified as a persistent organic pollutant (POP) in 2004. It is still found in the environment more than 30 years since their prohibition [1]. In Japan, dieldrin concentrations exceeding 0.02 ppm have been detected in cucumbers, and have also been detected in the soil at a maximum concentration of 2.6 ppm [2]. Therefore, contamination with dieldrin is still a serious environmental problem and an efficient remediation method is required. The aerobic degradation of organochlorine pesticides using bacteria and fungi has been reported [3–5]. Matsumura and Boush [6] isolated *Trichoderma viride* as a dieldrin degrader from soil that had been heavily contaminated with various insecticides. Wedemeyer [7] also described the degradation of dieldrin *in vitro* by *Aerobacter aerogenes*. Furthermore, several microorganisms, such as *Phrebia* sp. [8], *Mucor* sp. [9] and *Burkholderia* sp. [3], which are able to degrade dieldrin, were isolated after 2008. However, only two bacterial strains have been identified as being able to degrade dieldrin. This would suggest that aerobic bacteria that are able to degrade dieldrin are difficult to isolate because of the bioavailability of dieldrin. In a past study, Takagi et al. [4] isolated hexachlorobenzene (HCB)-degrading bacteria using pentachloronitrobenzene (PCNB) as an enrichment substrate. PCNB is more soluble in water than HCB; therefore, the bioavailability of PCNB in soil is higher than HCB. In another study, Matsumoto et al. [3] isolated dieldrin-degrading bacteria using 1,2-epoxycyclo hexane (ECH) as a structural analog. Structural analogs, such as PCNB and ECH, are expected to be useful substrates for isolation of dieldrin biodegraders. Moreover, there have been some reports that dieldrin is converted into aldrin *trans*-diol by microorganisms [6,7,9]. Aldrin *trans*-diol exhibits approximately 10-fold greater water-solubility than dieldrin. The solubility of dieldrin in water is extremely low (0.1–0.25 mg/l). To enrich dieldrin-degrading bacteria we used aldrin *trans*-diol as an enrichment substrate. In

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the present study, we tried to isolate dieldrin-degrading bacteria using a soil–charcoal perfusion method with aldrin *trans*-diol as an analog substrate. We successfully obtained novel aerobic bacteria that could effectively degrade dieldrin in soil.

2. Materials and methods

2.1. Soil samples

Three soil samples were used for the isolation of dieldrin-degrading bacteria; these were collected from different agricultural sites (two in Kagoshima prefecture, one in Ehime prefecture) with a history of repeated endosulfan application [10].

2.2. Chemicals

Dieldrin ((1aα,2aα,6aα,7aα)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2β,7β:3α,6α-dimethanonaphth[2,3-b]oxirene), heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7atetrahydro-4,7-methano-1*H*-indene), heptachlor epoxide (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1*H*-indene), endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin3-oxide), and endosulfan sulfate (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin3,3-dioxide) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Chlordecone (1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-2*H*-1,3,4-(methanetriyl)cyclobuta[*cd*]pentalen-2-one) was purchased from Riedel-de Haen (Germany). (±)-Aldrin *trans*-diol ((±)-1,2,3,4,10,10-hexachloro-1,4,4aβ,5,6,7,8,8aβ-octahydro-1β,4β:5α,8α-dimethanonaphthalene-6β, 7α-diol) was synthesized as described by Kataoka et al. [9]. Dihydrochlordendicarboxylic acid (DHCDS) was synthesized from aldrin using HNO_3 [11] or a ^{14}C-labeled form using $KMnO_4$ [12]. We chose milder stepwise oxidation, because direct oxidation by $KMnO_4$ resulted in a low yield (50%). Lemieux–Johnson oxidation of aldrin afforded dihydrochlordenedial, which was then converted to DHCDS by Jones oxidation. The yield of aldrindicarboxylic acid ((1*R*,3*S*,3a*R*,4*R*,7*S*,7a*S*)-4,5,6,7,8,8-hexachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene-1,3-dicarboxylic acid) was 99% over two steps.

Dichlorochlordenedial. A suspension of aldrin (MW: 364.91, 2.14 g, 5.86 mmol), a solution of OsO_4 in *t*-BuOH (1%, 1.5 ml), *N*-methylmorpholine *N*-oxide (2.0 g, 20 mmol) and $NaIO_4$ (9.0 g, 42 mmol) in dioxane–H_2O (7:4, 55 ml) was stirred at 20 °C for 6 h. The mixture was diluted with EtOAc and washed with a sat. aq. $Na_2S_2O_3$ solution, H_2O and brine, dried with $MgSO_4$ and concentrated *in vacuo* to give crude dial (2.5 g) as a pale yellow oil. A small portion was chromatographed on silica gel (hexane/EtOAc = 10:1∼2:1 and $CHCl_3$/MeOH = 20:1) for further purification. R_f 0.24 (hexane/EtOAc = 3:1). IR (ATR, Zn–Se) ν_{max}^{-1}: 2961 (w), 1727 (s, C=O), 1601 (s), 1033 (s), 756 (vs), 694 (vs), 601 (s). ^{13}C-NMR (125 MHz, $CDCl_3$) d: 27.54, 51.04, 52.87, 80.84 (C-4), 109.73 (C-8), 131.23 (C-5), 198.43 (CHO). FAB-MS *m/z*: 397 $[M(^{35}Cl_6)+H]^+$.

Aldrin dicarboxylic acid. To a solution of crude dihydrochlordenedial in acetone (20 ml), 8 mol/l Jones' reagent (CrO_3 in H_2SO_4, 1.4 ml) was added dropwise at 0 °C. To this was added *i*-PrOH and then a 5% aq. $NaHSO_3$ solution. The mixture was extracted four times with ethyl acetate. The organic layer was combined and extracted three times with a 15% aq. NaOH solution. Then the combined aqueous layer was acidified with a 2 mol/l aq. HCl solution and extracted four times with ethyl acetate. The combined organic layer was concentrated *in vacuo* to give DHCDS as a crystalline powder (2.50 g, 5.83 mmol, 99%). Recrystallization from hexane to acetone gave pure DHCDS, mp 263 °C (dec.) IR (ATR, Zn–Se) $\nu_{max}{}^{-1}$: 2890 (br. m), 1714 (s, C = O), 1595 (m), 1431 (m), 1281 (s), 1212 (s), 899 (s), 813 (s), 703 (s), 687 (m). ^1H NMR (600 MHz, CD_3OD) δ: 2.25 (1H, dt, *J* = 13.1, 9.2 Hz), 2.31 (1H, dt, *J* = 13.1, 7.4 Hz), 2.67 (2H, m), 3.81 (2H, m). HR-FAB-MS *m/z*: 426.8635 ($[M+H]^+$, calcd. for $C_{12}H_9O_4{}^{35}Cl_4$: 426.8632).

2.3. Growth media for microorganisms

Difco™R2A agar medium was from Becton Dickinson (Franklin Lakes, NJ, USA). The YM agar medium used in the present study was composed of 4.0 g/l yeast extract, 10.0 g/l malt extract, 4.0 g/l glucose and 20.0 g/l of agar. The mineral salt medium (MM) used contained 2.4 g/l Na_2HPO_4 $12H_2O$ and 1.0 g/l of KH_2PO_4. The medium was autoclaved and then supplemented with $MgSO_4{\cdot}7H_2O$ (20 mg/l) and a trace element solution (10 ml/l). The trace element solution was modified from Yanze-Kontchou and Gschwind [13] (200 mg/l $FeSO_4{\cdot}7H_2O$, 10 mg/l $ZnSO_4{\cdot}7H_2O$, 5 mg/l $MnSO_4{\cdot}H_2O$, 30 mg/l H_3BO_3, 24 mg/l $CoSO_4{\cdot}7H_2O$, 5 mg/l $CuSO_4{\cdot}5H_2O$, 5 mg/l $NiSO_4{\cdot}7H_2O$, 5 mg/l Na_2MoO_4, and 50 mg/l $Ca(OH)_2$. MM was supplemented with a 1/1000 volume of filter-sterilized acetone solution of organochlorine pesticides after autoclaving, unless otherwise noted. During cultivation, all of the agar plates were maintained in plastic bags to prevent desiccation.

2.4. Analytical methods

Aldrin *trans*-diol and aldrindicarboxylic acid were monitored by high-pressure liquid chromatography (HPLC; Hewlett–Packard series 1100) equipped with a photodiode array (PDA) detector set at 220 nm). A Wakosil II 5C18 RS column (250 × 4.6 mm internal diameter; Wako) with a mobile phase of 70% acetonitrile in 0.1% phosphoric acid was used at a 1.0 ml/min flow rate and 40 °C. Dieldrin was detected with an electron capture detector-gas chromatograph (ECD-GC; HP 6890; Hewlett–Packard, Palo Alto, CA, USA). A capillary-type column with a splitless injector (HP-50+ with 50% Ph Me silicone gum), internal diameter of 0.53 mm, a length of 15 m, and film thickness of 1 μm was used. The oven, injector port, and detector were maintained at temperatures of 180 °C, 250 °C, and 300 °C, respectively. Concentration of chloride ions was measured by ion chromatography (761 Compact IC; Metrohm). A TSK gel Super IC-Anion column (Tosoh) and a TSK guard column Super IC-AP (Tosoh) with a mobile phase of 1.8 mM Na_2CO_3 and 1.7 mM $NaHPO_4$ was used at a 1.0 ml min^{-1} flow rate and 32 °C. Aldrindicarboxylic acid was detected using a Waters Micromass ZQ-4000 LC/MS system equipped with a PDA detector set at 220 nm. A Wakosil II 5C18 RS column (250 × 4.6 mm internal diameter; Wako) with a mobile phase of 70% acetonitrile in 0.2% acetic acid aqueous solution was used at a 0.5 ml/min flow rate and 40 °C. The MS system was equipped with an electrospray chamber in the negative-ion mode, the capillary voltage was 3.2 kV, cone voltage was 10 V, extractor voltage was 3.0 V, RF lens voltage was 0.2 V, source temperature was 120 °C; and the desolvation temperature was 350 °C. Gas chromatography–mass spectrometry (GC–MS) was performed with a HP 6890 GC system linked to a HP 5973 mass selective detector and a 30-m fused DB-5MS column (J&W Scientific, Folsom, CA). The oven temperature was programmed to increase from 80 to 320 °C at 20 °C/min.

2.5. Enrichment culture

Enrichment of dieldrin-degrading bacteria was performed using the original soil–charcoal perfusion method [4,14]. The soil sample (40 g dry weight) was mixed with autoclaved charcoal A100 (2 g, grain size 5–10 mm, BET specific surface area of 100 m^2/g, pH 7.8; Toyo Denka Kogyo, Kochi, Japan) as a microhabitat and aldrin *trans*-diol. The soil–charcoal mixture was washed twice with sterilized water to remove chloride ions

and then set in a perfusion apparatus [15]. The surface of the soil–charcoal layer was covered with a glass microfiber filter. The first enrichment culture was carried out in the dark at 25 °C by circulating 300 ml of MM containing 5 mg/l aldrin *trans*-diol. The medium was circulated using an air pump through the soil–charcoal layer in the perfusion apparatus. The perfusion rate of the medium was adequately controlled by the air pump, and smooth leaching was maintained. The medium was replaced periodically. Aliquots of the culture were centrifuged at 19,000 *g* at ambient temperature for 10 min. The aldrin *trans*-diol and aldrindicarboxylic acid concentrations were determined by HPLC. After aldrin *trans*-diol degradation in the first enrichment culture, 0.25 g of the charcoal was transferred to another apparatus with 10.0 g of newly autoclaved charcoal A100. Further enrichment and purification were performed by circulating 300 ml of MM containing 5 mg/l of aldrin *trans*-diol with 200 mg of pyruvic acid as a carbon source. The pH of the enrichment culture was maintained at 7.0.

2.6. Isolation of aldrin trans-diol-degrading bacteria

The enriched charcoal (1 g) was crushed and suspended in 50 mM phosphate buffer (pH 7.0); the same buffer was added to the suspension to formulate a 10^{-5}-fold dilution. The diluted suspension was then inoculated on an R2A agar plate. The colonies formed on the R2A agar plate were isolated and tested for aldrin *trans*-diol degradation in MM.

2.7. Identification of aldrin trans-diol degrading bacteria

To characterize the aldrin *trans*-diol-degrading bacterium, morphology and physiology of cells grown on YM agar for 2 week at 30 °C were examined by Techno Suruga Laboratory Co., Ltd. (Shizuoka, Japan). Total bacterial DNA was extracted using a FastDNA SPIN Kit (QBioGene) and purified with phenol–chloroform. The 16S rRNA gene was amplified using primers 9f (5′-GAG TTT GAT CCT GGC TCA G-3′), 1541r (5′-AAG GAG GTG ATC CAG CC-

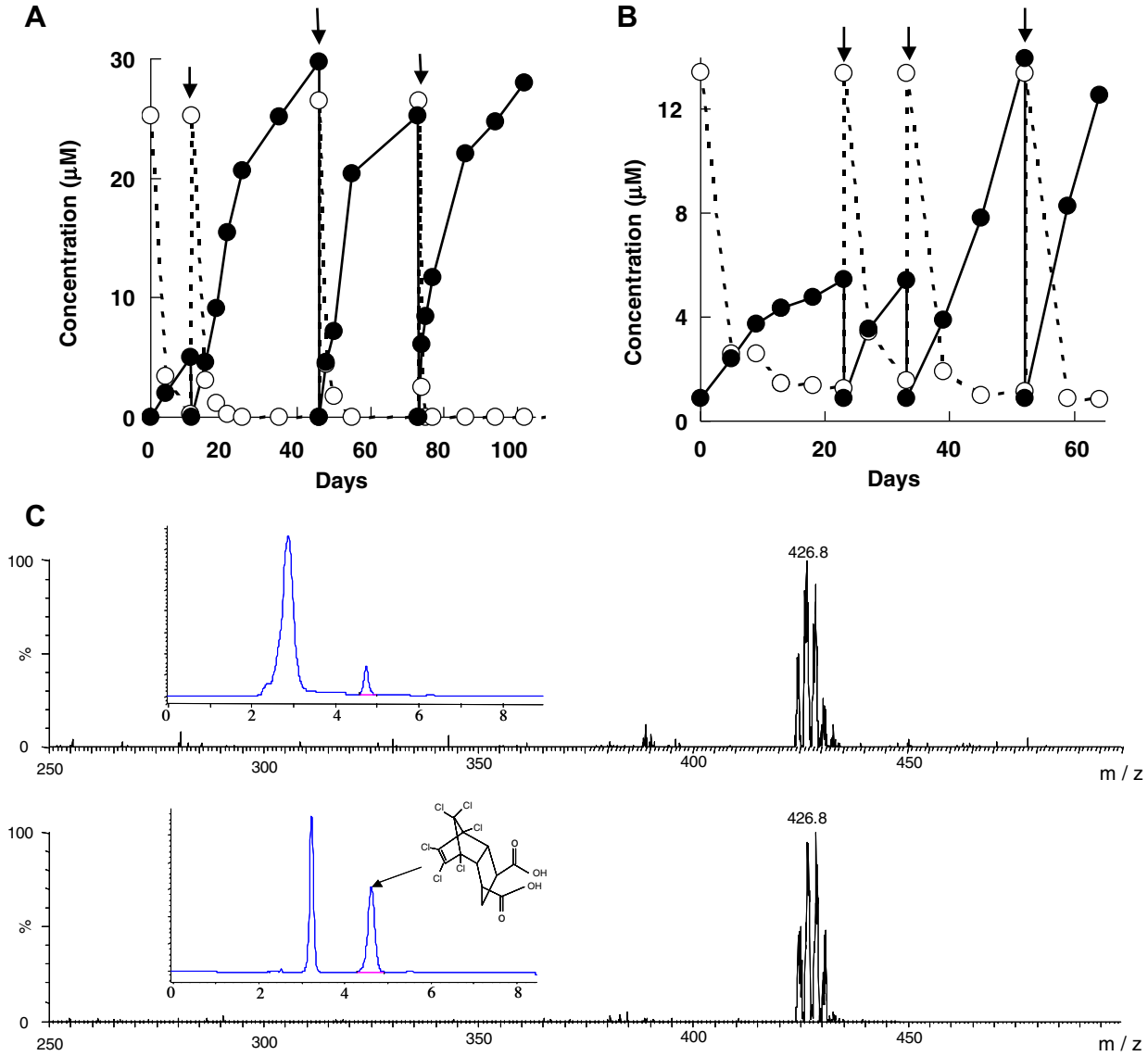

Fig. 1. (a) Time course of the first enrichment culture using a soil–charcoal perfusion method containing aldrin *trans*-diol. (b) Time course of the secondary enrichment culture using a charcoal perfusion method. Changes in concentration of aldrin *trans*-diol (○) and aldrin dicarboxylic acid (●) in the culture supernatant are indicated. The arrows indicate the time points where replacement of perfusion fluid occurred. (c) The HPLC chromatograph and MS spectrum for aldrindicarboxylic acid. Top: a sample of aldrindicarboxylic acid. Bottom: authentic standard of aldrindicarboxylic acid synthesized in this study. The peak retention time and MS fragment of the metabolite matched the standard.

3′). Polymerase chain reactions (PCR) were processed on a TaKaRa PCR thermal cycler (TaKaRa) according to the following profile: one cycle of 5 min at 95 °C; followed by 30 cycles of 30 s at 95 °C; 30 s at 58 °C; 1 min at 72 °C; and a final cycle of 7 min at 72 °C. The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen) and subjected to cycle sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied-Biosystems). The following 16S rRNA primers were used: 9f, 1541r, 515f (5′-GTG CCA GCA GCC GCG GT-3′), 785f (5′-GCA TTA GAT ACC CTG GTA GTC-3′), 907r (5′-CCA TCA ATT CCT TTA AGT TT-3′), and 1242r (5′-CCA TTG TAG CAC GTG T-3′). The reaction products were purified with Performa DTR Gel Filtration Cartridges (Edge Bio) and analyzed on a 3100 Genetic Analyzer (Applied-Biosystems). The obtained 16S rRNA sequence was compared with the bacterial sequences in the GenBank database. A phylogenetic dendrogram was constructed by the neighbor joining method with CLUSTAL X and njplotWIN95 [16].

2.8. Degradation of dieldrin

Mineral salt medium (10 ml) containing 5 mg/l dieldrin and 200 mg/l pyruvic acid was added to a 50-ml Erlenmeyer flask. The media were inoculated with a suspension of the aldrin *trans*-diol-degrading bacteria surface culture from the YM agar plate. The flask cultures were shaken at 210 rpm on a rotary shaker at 30 °C in the dark for 10 days. Triplicate flasks were withdrawn periodically, and cell growth was monitored by measuring the optical density at 600 nm (OD_{600}) with a V-630bio spectrophotometer (JASCO). As a control, cultures were killed by autoclaving (121 °C, 15 min). Aliquots (1.0 ml) of culture fluid were removed and subject to centrifugation at 19,000*g* for 10 min. Concentration of chloride ions in the supernatant was determined by ion chromatography. The remaining culture fluid was extracted with 30 ml of hexane. Dieldrin was analyzed by GC/ECD. The recovery rate of dieldrin in the control cultures was found to be 90–100%, which indicated that the extraction was efficient, and the analysis deviation was small. The time course degradation experiment for dieldrin-degrading bacteria was performed in triplicate. Experiments investigating the degradation of α-endosulfan, β-endosulfan, endosulfan sulfate, heptachlor, heptachlor epoxide and chlordecone by aldrin *trans*-diol degrading bacteria were also analyzed by GC/ECD. Degradation of chlordecone was analyzed by GC/MS.

3. Results

3.1. Enrichment and isolation of aldrin trans-diol-degrading bacteria

At the beginning of enrichment, the generation of aldrindicarboxylic acid in the perfusion culture was detected 10 days of circulation, while aldrin *trans*-diol was not detected (Fig. 1A). During 100 days of circulation and three exchanges of the medium, the generation rate of aldrindicarboxylic acid increased (Fig. 1A). The charcoal was transferred to the charcoal perfusion (secondary enrichment) culture after 100 days of circulation. The aldrin *trans*-diol-degrading bacteria were highly enriched in the charcoal during the second enrichment (Fig. 1B). After 62 days of circulation and three exchanges of the medium, the enriched charcoal was harvested to carry out subsequent colony isolation. Several types of colonies having different morphology were observed on the R2A agar. The aldrin *trans*-diol-degrading abilities of the individual isolates were examined in tube cultures and subsequent flask cultures. An isolate that showed a distinctive decrease of aldrin *trans*-diol (total 10 mg/l) after 20 days cultivation in a flask culture was obtained, and named strain KSF27.

3.2. Identification of strain KSF27

Strain KSF27 was Gram-positive, with a colony size ranging from 0.5 to 1.0 mm. The surface shape of colony was flocculent, and the surface of the aerial hypha was orange–white on the YM agar plate culture. Tests relating to water solubility, melanin pigment, gelatine liquefaction, starch hydrolysis, nitrate reduction and powdered skim milk peptonization were negative, negative, negative, positive, negative, and negative, respectively. The temperature range for growth was 20–37 °C. Non-halotolerance, glucose, D-mannitol, D-fructose and inositol were assimilated, while

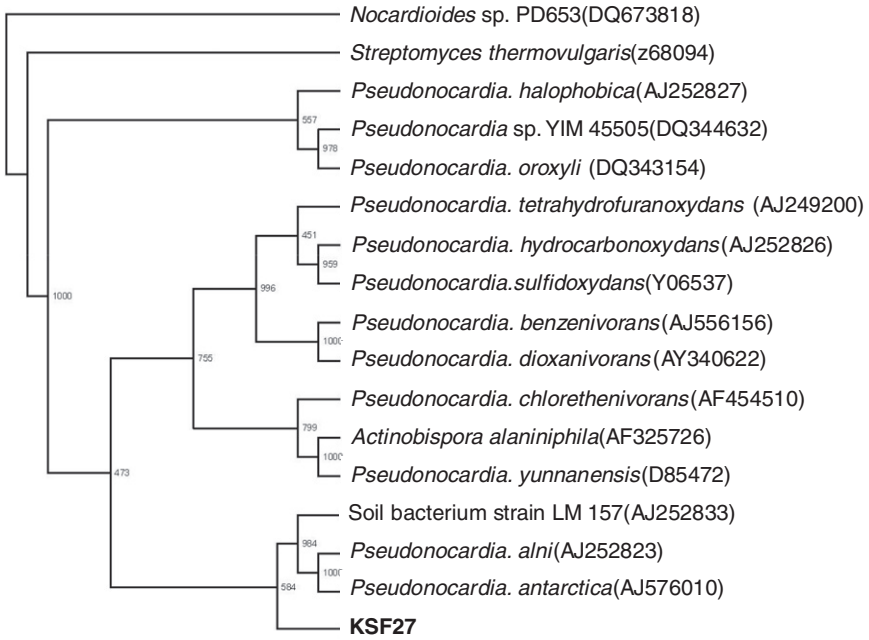

Fig. 2. Phylogenetic relationships of strain KSF27 isolated in this study and related species. The phylogenetic tree of 16S rRNA sequences was generated by the neighbor-joining method. The tree was tested for support by performing bootstrap resampling (1000 replicates). The bootstrap values are given at each branch, and Genbank accession numbers of each sequence employed are in parentheses.

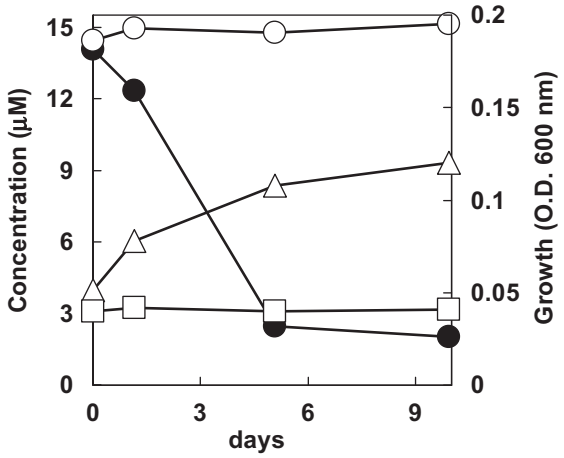

Fig. 3. Aerobic degradation of dieldrin by *Pseudonocardia* sp. strain KSF27. The triangles and squares indicate the OD_{600} of the KSF27 cultures and heat-killed controls, respectively. The shaded and unshaded circles indicate the dieldrin concentration in KSF27 cultures and the heat-killed controls, respectively.

L-rhamnose, L-arabinose, raffinose, sucrose and D-xylose were not assimilated. The sequence of strain KSF27 (1517 nt; GenBank accession No. AB630184) was compared with other bacterial nucleotide sequences in GenBank. Strain KSF27 exhibited a high sequence similarity *Pseudonocardia* spp. The highest sequence similarity (95%) of the 16S rRNA gene was found in *Pseudonocardia alaniniphila* strain YIM 16303 (EU722519). The 16S rRNA of strain KSF27 was aligned with those of the representative strains of the Actinomycetales, and a phylogenetic dendrogram was constructed (Fig. 2). Based upon the genetical and morphological characteristics, strain KSF27 is a new species of the genus *Pseudonocardia*, designated *Pseudonocardia* sp. strain KSF27.

3.3. Degradation of dieldrin by strain KSF27

The initial concentration of dieldrin in the aerobic culture of strain KSF27 was 14.06 μM. This decreased to 2.01 μM after a 10-day incubation. Approximately 85% of dieldrin was degraded over the initial 5 days of culture. Dieldrin degradation then continued at a slower rate. An apparent increase of OD_{600} was obtained after 10 days in culture (Fig. 3). Chloride ions were not detected.

3.4. Degradation of other organochlorine pesticides by strain KSF27

The degradation capability of strain KSF27 was examined using α-endosulfan, β-endosulfan, endosulfan sulfate, heptachlor, heptachlor epoxide and chlordecone. We observed that strain KSF27 degraded all of the persistent compounds. The concentrations of α-endosulfan, β-endosulfan and endosulfan sulfate were reduced to 0.20 μmol/l (97.5%), 0.15 μmol/l (97.8%) and 2.70 μmol/l (71.8%), respectively. Heptachlor, heptachlor epoxide and chlordecone concentrations were reduced to 0.32 μmol/l (83.9%), 6.08 μmol/l (53.4%) and 0.36 μmol/l (87.2%), respectively (Table 1).

4. Discussion

A new dieldrin-degrading bacterium was isolated from soil. This strain degraded the amount of dieldrin by 90% following a 10-day incubation (Fig. 3). Enrichment cultures using the soil–charcoal perfusion system are an effective way to obtain microorganisms that degrade recalcitrant compounds [4,17,18]. However, the extremely low solubility of dieldrin in water (0.1–0.25 mg/l) makes it difficult to apply this system. Therefore, the structural analog, aldrin *trans*-diol, with a higher solubility of 2.5 mg/l in water was adapted in the soil–charcoal perfusion system for enriching desired microorganisms. To date, the soil–charcoal perfusion has only been a useful method for pollutants utilizing bacteria [4,17]. In the present study, the enrichment culture was added not only aldrin *trans*-diol but also pyruvic acid as a carbon source in this study. Consequently, this aldrin *trans*-diol-degrading bacterium is co-metabolic one which utilize pyruvic acid as a carbon source for growth (Fig. 3). Rapid degradation of aldrin *trans*-diol was observed at the beginning of circulation and after replacing the perfusion medium. It is likely this was caused by enrichment of aldrin *trans*-diol-degrading bacteria in the charcoal. The metabolite of aldrin *trans*-diol, aldrindicarboxylic acid, was detected by HPLC/MS (Fig. 1C); however chloride ions were not detected in this study. This would suggest that this bacterium is unable to de-chlorinate dieldrin. Sequencing analysis of the 16S rRNA showed that this strain belonged to *Pseudonocardia*. The sequence of the 16S rRNA gene was most similar (95%) to that found in *P. alaniniphila* strain YIM16303 (EU722519). Based upon the phylogenetic taxonomic data, strain KSF27 is a new species of the genus *Pseudonocardia*, and has been designated as *Pseudonocardia* sp. strain KSF27. We detected aldrindicarboxyric acid from the initial metabolite of aldrin *trans*-diol by HPLC/MS. The full scan mass spectra of aldrindicarboxyric acid showed a molecular ion peak at *m/z* 427 (Fig. 1C). The retention time and molecular ion peak of aldrindicarboxyric acid matched an authentic standard which we synthesized in this study. We suggest that strain KSF27 converted

Table 1
Degradation of organochlorine pesticide by strain KSF27.

	KSF27	Control	Degradation (%)
Dieldrin	3.76 ± 0.97	14.09 ± 1.02	73.3
α-Endosulfan	0.20 ± 0.10	8.147 ± 0.83	97.5
β-Endosulfan	0.15 ± 0.10	6.871 ± 1.73	97.8
Endosulfan sulfate	2.70 ± 0.60	9.587 ± 0.88	71.8
Heptachlor	0.32 ± 0.03	1.988 ± 0.31	83.9
Heptachlor epoxide	6.08 ± 0.46	13.07 ± 1.45	53.4
Chlordecone	0.36 ± 0.12	2.822 ± 0.59	87.2

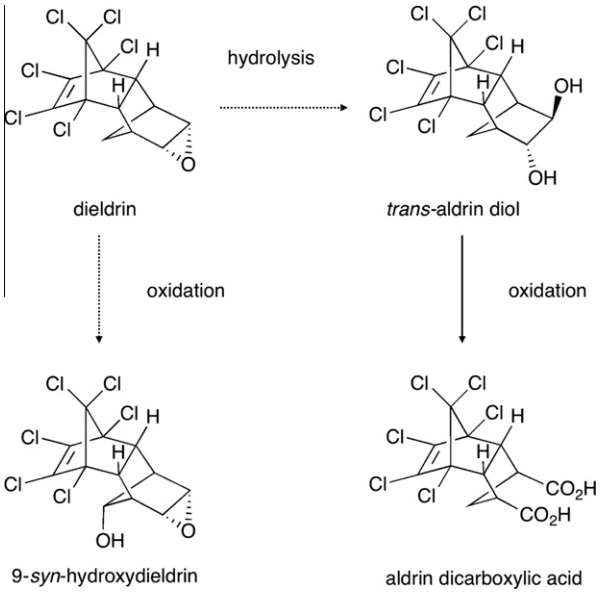

Fig. 4. Proposed metabolic pathway of dieldrin transformation by strain KSF27. Solid arrows indicate the major metabolic pathway where aldrin-*trans* diol is the initial substrate; broken arrows indicate a presumptive pathway. 9-*syn*-hydroxydieldrin and aldrin *trans*-diol were detected by Kamei et al. [8] and Wedemeyer [7], respectively.

dieldrin to aldrindicarboxyric acid via aldrin *trans*-diol (Fig. 4). There are some reports regarding the degradation of 1,4-dioxane and tetrahydrofuran by *Pseudonocardia* spp. [19,20]. The initial monooxidation of 1,4-dioxane likely results in production of 2-hydroxy-1,4-dioxane. This compound could be oxidized through a hydroxyl aldehyde intermediate to 2-hydroxyethoxyacetic acid [20]. Tetrahydrofuran is also oxidized by hydroxylation of an ether-bond-associated carbon atom by monooxygenases, resulting in ether bond cleavage to form 4-hydroxybutyrate [19]. Moreover, we revealed that strain KSF27 was able to degrade dieldrin, endosulfan, endosulfan sulfate, heptachlor, heptachlor epoxide and chlorodecone (Table 1). Therefore, we conclude that strain KSF-27 possesses the ability to degrade a broad range of recalcitrant organochlorinated pollutants.

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