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Biodegradation of 1,4-dioxane by a *Flavobacterium*

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Abstract A dioxane-degrading consortium was enriched from soil obtained from a contaminated groundwater plume. The enriched consortium did not use dioxane as the sole source of carbon and energy but co-metabolized dioxane in the presence of tetrahydrofuran (THF). THF and dioxane concentrations up to 1000 ppm were degraded by the enriched consortium in about 2 weeks with a longer lag phase observable at 1000 ppm. Three colonies from the enriched consortium were then obtained on agar plates containing basal salts and glucose as the carbon source. Only one of the three colonies was capable of dioxane degradation. Further enrichment of this colony in liquid media led to a pure culture that grew on glucose and co-metabolically degraded dioxane after THF degradation. The rate and extent of dioxane degradation of this isolate increased with increasing THF concentration. This isolate was subsequently identified as a *Flavobacterium* by 16S rDNA sequencing. Using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis of microbial populations, *Flavobacterium* was determined to be the dominant species in the enriched consortium and was distinct from the two other colonies that did not degrade dioxane. This is the first report of a dioxane-degrading *Flavobacterium* which is phylogenetically distinct from any previously identified dioxane degrader.

Keywords Dioxane · Biodegradation · Flavobacterium · DGGE · PCR

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Introduction

The cyclic ether 1,4-dioxane (dioxane) is used primarily to stabilize chlorinated solvents and is considered a potential human carcinogen. In the past three decades, approximately 0.5–1.0 million pounds of dioxane were released in the United States each year (Toxic Release Inventory 1988–2005), and it has been detected in groundwater samples (Zenker et al. 2000). The guidelines for maximum contaminant level (MCL) in drinking water ranged from 3 parts per billion (ppb) in California to 85 ppb in Michigan (Duncan et al. 2004). Dioxane is recalcitrant, chemically stable, miscible with water, and has a low Henry's Law constant. Such properties make natural attenuation of dioxane in contaminated groundwater insignificant (Zenker et al. 2004; Mahendra and Alvarez-Cohen 2006) and physical separation difficult (Zenker et al. 2004).

Physical methods such as ultrasonic decomposition (Beckett and Hua 2003) have been evaluated and advanced oxidation processes (AOPs) using hydrogen peroxide, ozone, and UV photo-oxidation are proven technologies for dioxane treatment (Zenker et al. 2003). The operating costs of AOP are normally high with additional costs incurred when used in combination with a "pump and treat" approach (Mahendra and Alvarez-Cohen 2006).

Phytoremediation is another option being assessed. Hybrid poplar trees have been used to remediate dioxane in hydroponic and soil experiments (Aitchison et al. 2000). Although Kelley et al. (2001) found that a dioxane degrader, *Amycolata* sp. CB1190, enhanced dioxane removal by hybrid poplar trees planted in soil, the majority of dioxane loss was due to transpiration. When transpired to air, the half-life of dioxane was estimated to be 6.7–9.6 h as a result of photo-degradation (OPPT Chemical Fact Sheets-1,4-dioxane 1995).

Microbial degradation of dioxane is an attractive remediation approach because dioxane can be mineralized if used as the sole source of carbon and energy (Parales et al. 1994) or as a co-metabolite (Zenker et al. 2000). However, dioxane has been reported to be recalcitrant to biodegradation. It is poorly biodegraded probably because its stable ether bond requires 360 kJ/mol of energy for cleavage (Zenker et al. 2004). To date, only a few aerobic microorganisms have exhibited dioxane-degrading capabilities. *Rhodococcus ruber* 219 (Bernhardt and Diekmann 1991; Bock et al. 1996), *Amycolata* CB1190 (later identified as *Pseudonocardia dioxanivorans* CB1190) (Parales et al. 1994; Mahendra and Alvarez-Cohen 2005), *Mycobacterium* sp.PH-06 (Kim et al. 2009) and a fungus, *Cordyceps sinensis* (Nakamiya et al. 2005) can use dioxane as a sole source of carbon and energy. *Mycobacterium vaccae* JOB 5 (Burback and Perry 1993), *Pseudonocardia* K1 (Kohlweyer et al. 2000), *Pseudonocardia* sp. ENV478 (Vainberg et al. 2006), *Graphium* sp. (Skinner et al. 2009) and a mixed culture (Zenker et al. 2000) have been identified as micro-organisms that degraded dioxane but were unable to grow on it.

Tetrahydrofuran (THF), a structural analog of dioxane, has been used to enrich for dioxane degraders (Parales et al. 1994; Zenker et al. 2000; Vainberg et al. 2006). *R. ruber* 219 grew faster on THF than on dioxane, and *P. dioxaneivorans* CB1190 was thought to be a THF-degrading mutant capable of using dioxane as the sole source of carbon (Parales et al. 1994). Since THF is also a significant environmental contaminant, its use should be minimized. Interestingly, there were reports of micro-organisms that did not require THF or other structural analogues to degrade dioxane. *Mycobacterium vaccae* JOB 5 (Burback and Perry 1993) enriched from propane, the propanotroph SL-D (Fam et al. 2005), and *Methylosinus trichosporium* OB3b (Mahendra and Alvarez-Cohen 2006) all degraded dioxane without using another dioxane-like carbon source. Mahendra and Alvarez-Cohen (2006) showed that methane monooxygenase could be a key enzyme in dioxane degradation. However, methane and propane are both of low water solubility, flammable gases, and growth would be low because of mass transfer problems.

The objective of this study was to isolate microorganism(s), from dioxane-contaminated soil, capable of degrading dioxane as the sole source of carbon or co-metabolically in the presence of THF. Since the isolate obtained was co-metabolic in terms of dioxane degradation, a strategy to minimize THF usage in culture preparation and dioxane degradation was examined. The identity of this dioxane-degrading microorganism was revealed by denaturing gradient gel electrophoresis (DGGE) and 16S rDNA sequencing.

Materials and methods

Microcosm growth medium

Basal salts medium (BSM) (Parales et al. 1994) was modified and used in the enrichment and culturing experiments. Each liter of media contained 100 mL BSM stock solution and 100 mL trace element solution. BSM stock solution contained per liter: 32.4 g K_2HPO_4, 10 g $NaH_2PO_4{\cdot}H_2O$, and 20 g NH_4Cl. The trace element solution contained per liter: 1.5 g nitrilotriacetic acid, 3.0 g $MgSO_4{\cdot}7H_2O$, 1.0 g NaCl, 0.1 g $FeSO_4{\cdot}7H_2O$, 0.1 g $CoCl_2{\cdot}6H_2O$, 0.132 g $CaCl_2{\cdot}2H_2O$, 0.1 g $ZnSO_4{\cdot}7H_2O$, 8.74 mg $CuSO_4{\cdot}5H_2O$, 10.0 mg $AlK(SO_4)_2{\cdot}12H_2O$, 10.0 mg H_3BO_3, 10 mg $Na_2MoO_4{\cdot}2H_2O$, 27.1 mg $NiCl_2{\cdot}6H_2O$, and 20.0 mg $Na_2WO_4{\cdot}2H_2O$.

Enrichment and culturing conditions

To obtain an enriched consortium, soil from a groundwater plume contaminated with dioxane was

added to 125 ml serum bottles containing 100 mL BSM and incubated at 25 ± 1°C and 200 rpm on an Innova rotary shaker. THF or dioxane (80–320 ppm (v/v)) was added as the sole source of carbon and energy. After degradation was detected, enrichments were serially transferred to fresh BSM medium containing 100 ppm each of THF and dioxane. The enriched consortium was propagated under similar conditions in Erlenmeyer flasks with 50 ml BSM and capped with a rubber stopper in all experiments employing THF or dioxane.

Dioxane degradation with THF by the enriched consortium

To investigate if dioxane can be used as a sole source of carbon and energy by the enriched consortium culture, 5 ml of the enriched consortium was added to 45 ml BSM with 100 ppm dioxane with or without 100 ppm THF. The effect of substrate concentration was determined by adding 5 ml of enriched mixed culture to 45 ml BSM containing THF and dioxane, each at 100, 300, and 1000 ppm. Abiotic controls were identical to the biotic microcosms except without inocula.

Obtaining isolates from the enriched consortium culture and dioxane degradation

The enriched consortium culture was streaked on BSM agar plates containing THF (300 ppm), dioxane (300 ppm), or glucose (10 g/l) as the sole source of carbon and energy. Three morphologically distinct colonies (designated Colonies A, B and C) found growing on the glucose plates were propagated separately for further studies. Each colony was cultured in BSM containing 10 g/l glucose. After centrifugation at 7663×*g* for 5 min in a Sorvall RC-5B centrifuge (Dupont), the resulting biomass was washed twice with sterile BSM to eliminate residual glucose, re-suspended in sterile BSM, and then used to inoculate BSM containing 100 ppm each of THF and dioxane. A dioxane-degrading culture from Colony A was maintained on BSM-glucose (10 g/l) and designated Isolate A.

Effect of THF concentration on dioxane degradation by isolate A

For these studies, the Isolate A (enriched from Colony A) was grown in 2 × 50 ml BSM-glucose (10 g/l) for 2 days, collected by centrifugation, and washed as described above. The resulting pellet was resuspended in a final volume of 10 ml BSM. One millilitre of this final suspension was then added to BSM containing 100 ppm dioxane and different concentrations of THF to test the effects of THF concentration on dioxane degradation.

Microbial population analysis

The enriched dioxane-degrading consortium, the three glucose colonies, and two replicates of the Isolate A were used for the microbial population analysis. One millilitre of each type of culture was centrifuged at 8000×*g* for 2 min in a bench top centrifuge (Eppendorf 5415C), washed twice with 0.5 ml deionized water, and resuspended in 50 µl deionized water for molecular characterization using polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) (Fortin et al. 2004). Concentrated cells (1 µl) were used as sources of PCR templates. The 16S rDNA fragments were amplified using a T1 thermocycler (Biometra), with an initial 10 cycles of 1 min denaturation at 94°C, 1 min annealing at 65°C stepping down 1° each cycle to 55°C, and 3 min extension at 72°C followed by 20 cycles of 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C. Each 50 µl PCR reaction contained 1 µl of concentrated cells, 8 µl of 1.25 mM dNTPs, 1 µl of each primer U341-GC2 and U758 (25 pmol) (Fortin et al. 2004), 4 µl 25 mM $MgCl_2$, 5 µl of 10× reaction buffer and 2.5 units of Taq polymerase (Fisher Scientific Ltd., Ottawa, Ontario). The amplified DNAs were separated using 8% (w/v), 0–80% denaturing gradient acrylamide gels in 1× TAE buffer. The gels were run using 80 V for 16 h at 60°C and stained for 10 min in 1× TAE containing SYBR Gold (Invitrogen, Burlington, Ontario).

For identification work, the 16S rDNA bands were excised and eluted with water at 4°C overnight, precipitated with ethanol, re-amplified for 25 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C using the U341 and 758 primers, and sequenced (Cortec, Kingston, Ontario). The 16S rDNA sequences were matched in GenBank using BLAST (Altschul et al. 1997) and the best matched sequences and those of previously reported dioxane or THF degraders between the U341 and U758 were retrieved. Phylogenetic and distance analysis were

performed with MEGA (4.0.2) and multiple sequence alignment was done with ClustalW which is integrated in MEGA (4.0.2) (Tamura et al. 2007). Bootstrap analysis was based on 1,000 replications.

Analytical methods

Dioxane and THF analysis

Various liquid culture samples were filtered through a 0.45 μm nitrocellulose filter (Millipore) and 1.5 μl of the filtrate with 200 ppm (v/v) 2-pentanol as the internal standard was injected into a Varian 3400 gas chromatograph equipped with a flame ionization detector and a 5 m guard column attached to a 30 m × 0.53 mm column (Restek stabilwax). The flow rates of helium, hydrogen and air were 4, 50, and 300 ml/min, respectively. The injector and detector temperature were held constant at 150 and 180°C, respectively, and the oven temperature profile was a 4 min hold at 60°C, followed by a ramp of 20°C/min ending at 150°C with a 1 min hold time.

Monooxygenase activity measurements

The monooxygenase activity of Isolate A, grown in both THF and dioxane, was determined in triplicate using a colorimetric assay (Brusseau et al. 1990) which quantified naphthol formation from naphthalene using 1% o-dianisidine tetrazotized dye and glacial acetic acid at an absorbance of 530 nm and related to a standard curve (0–48.6 μM l^{-1} 1-naphthol). *Methylosinus trichosporium* OB3b grown in a defined mineral salts medium with methane as the source of carbon and energy (Yu et al. 2009) was used as the positive control.

Results

Enrichment of a dioxane-degrading consortium

To enrich for dioxane-degrading microorganisms from soil contaminated with dioxane, nine microcosms were set up in BSM containing either dioxane or THF as the sole source of carbon and energy. After 9 months, dioxane degradation was not observed in any of the microcosms, and only one showed THF disappearance. The THF-degrading microcosm was serially transferred to BSM containing 100 ppm THF and 100 ppm dioxane until a mixed culture with dioxane and THF degradation capability was obtained. A typical dioxane and THF degradation curve is shown in Fig. 1. THF was completely degraded in about 5 days, and dioxane was degraded only when THF concentration reached a low level.

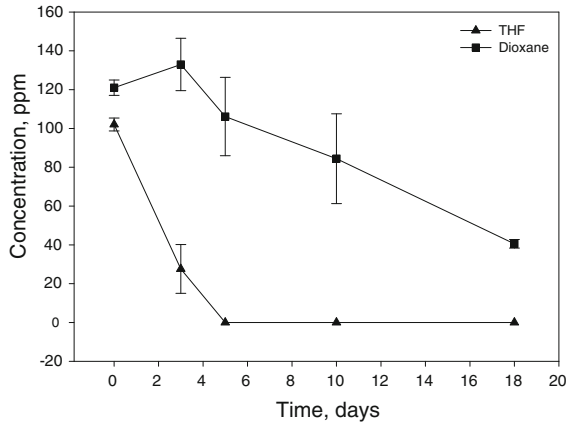

Fig. 1 Dioxane (*filled square*) and THF (*filled up-pointing triangle*) degradation by the enriched consortium. The *error bars* represent the standard deviation of three independent replicates

Co-metabolic degradation of dioxane by the enriched consortium

After the enriched consortium was obtained, the ability to degrade dioxane as the sole source of carbon and energy was tested. There was no significant loss of dioxane in the abiotic controls, and without THF, dioxane degradation was not observed in a three-week test period (Fig. 2). However, in biotic experiments supplemented with THF, dioxane degradation occurred after THF was degraded to low concentrations, with about 70% of the dioxane disappearing after 22 days (Fig. 2). This behavior appears to be similar to the pattern observed in Fig. 1.

Effect of THF and dioxane concentrations on the enriched consortium

The enriched consortium was grown on BSM containing THF and dioxane, each supplied at initial concentrations of 100, 300, or 1000 ppm. Dioxane and THF were degraded at all concentrations tested

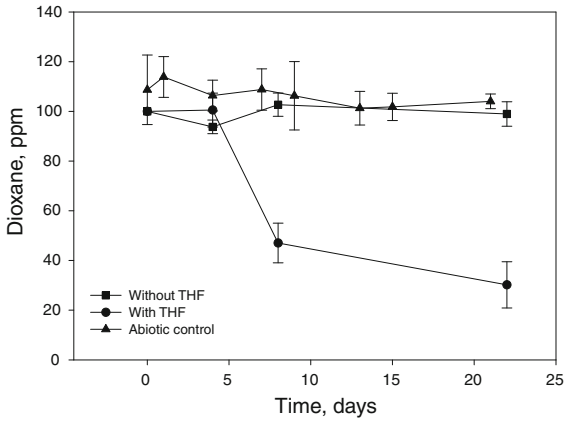

Fig. 2 Dioxane degradation by the enriched consortium was evaluated with (*filled circle*) or without (*filled square*) THF and compared to the abiotic control (*filled up-pointing triangle*) with THF. *Error bars* represent standard deviation of three independent replicates

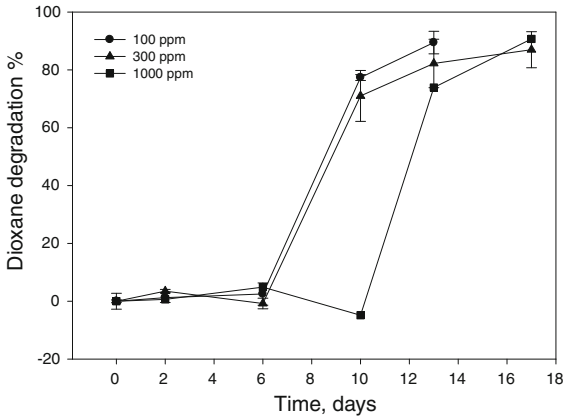

Fig. 3 Dioxane degradation at different initial concentrations. Both THF and dioxane were each initially at 100 (*filled circle*), 300 (*filled up-pointing triangle*), or 1000 (*filled square*) ppm. *Error bars* at 100 and 300 ppm represent the average of two replicates

(Fig. 3). The extent of dioxane degradation at all concentrations was similar with 90% dioxane degraded in about 2 weeks. During the degradation phase, the maximum dioxane degradation rate increased with increasing initial dioxane concentration. These rates were calculated to be 12.8, 35.6, and 64.3 ppm per day at 100, 300, and 1000 ppm of initial dioxane concentration respectively. However, the lag phase increased from 6 days at 100 and 300 ppm to 10 days at 1000 ppm. A similar result was reported for *Pseudonocardia* sp. strain K1 (Kohlweyer et al. 2000).

Preparation and subsequent dioxane degradation assessment of isolates

To obtain isolates of the dioxane degraders, the enriched mixed culture was plated on BSM agar containing either dioxane, THF, or glucose as the sole source of carbon and energy. There was no observable growth on dioxane. Growth on THF was slow with colonies too small to handle. On the glucose plates, there were three distinct colony morphologies observed (designated Colonies A, B and C). Colony A had a light brown appearance and were smaller than Colonies B and C. All three were grown separately in BSM liquid medium containing 10 g/l glucose, centrifuged, washed with BSM, and then tested for dioxane-degrading capabilities. None of the three colonies degraded dioxane alone. In the presence of THF, only Colony A degraded dioxane which decreased to below the detection limit in about 20 days (Fig. 4). Colony A also grew faster on glucose than on THF and dioxane and achieved a higher cell density. When grown on glucose, it appeared as a homogeneous suspension, but formed white clumps when grown on THF and dioxane. Similar observations were reported for *Pseudonorcardia* sp. strain ENV478 (Vainberg et al. 2006).

After growing on THF and dioxane, Colony A (2 ml) was transferred back to BSM-glucose (10 g/l) and maintained as a dioxane-degrading culture. The dioxane-degrading capability was re-tested in fresh BSM containing 100 ppm dioxane with or without

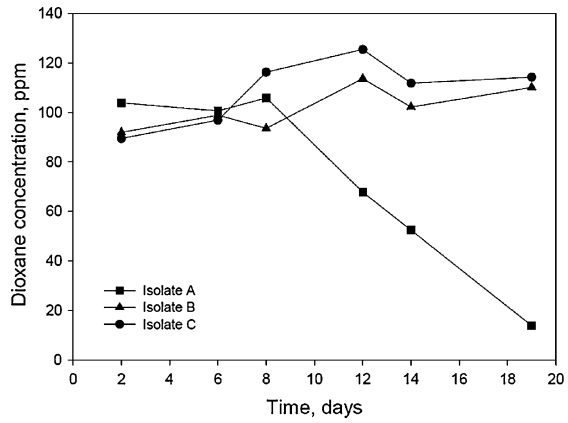

Fig. 4 Dioxane degradation of three colonies obtained from the dioxane-degrading consortium on BSM-glucose agar plates. The three different colonies named Colonies A (*filled square*), B (*filled up-pointing triangle*), and C (*filled circle*), were grown on BSM with 10 g/l glucose, and then transferred to fresh BSM containing about 100 ppm THF and 100 ppm dioxane

THF. Again, this culture did not use dioxane as the sole source of carbon and energy, but co-metabolically degraded dioxane with THF. Dioxane degradation was very stable after five transfers between glucose and THF/dioxane and this enriched culture was named Isolate A. It had no detectable monooxygenase activity using naphthalene as the substrate.

Microbial population analysis

The enriched consortium, Colonies A, B, and C from glucose plates, and two replicates of the Isolate A which was maintained in BSM-glucose medium were each analyzed using PCR and DGGE (Fig. 5). Four major bands were seen in the enriched consortium (lane 5). The bands from Colonies B and C (lanes 4 and 6, respectively) were not major bands in the enriched consortium (lane 5). Neither of these colonies, B or C, degraded dioxane (Fig. 4) and they were probably minor components of the enriched consortium but proliferated well on glucose. However, Colony A (lane 1) degraded dioxane (Fig. 4) and had a major band that matched the dominant band of the enriched consortium (compare lanes 1 and 5 in Fig. 5). When an attempt was made to obtain a pure culture from Colony A by transferring it several times in dioxane and THF in BSM, Isolate A was obtained. This had a single band which was clearly the dominant organism in the enriched culture (compare lanes 2 and 3 with 5). The two smaller bands were probably lost because those organisms did not use THF or dioxane as a source of carbon and energy.

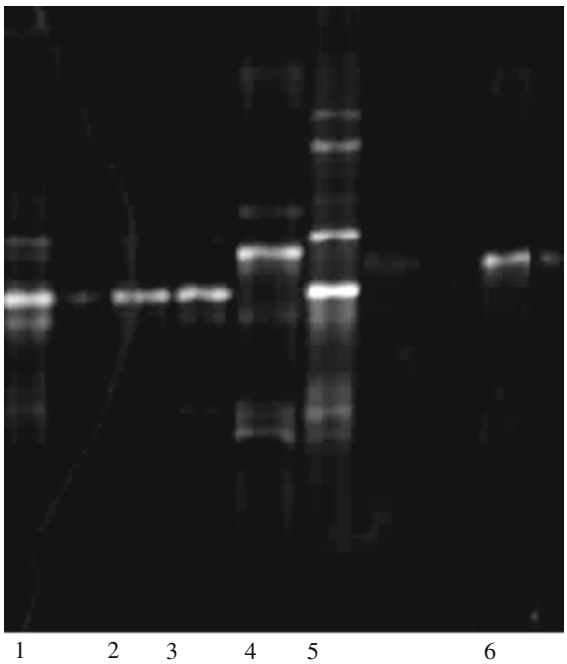

Fig. 5 DGGE analysis of the microbial populations. The 16S rDNA of the enriched consortium, Colonies A, B, and C from agar plates, and two replicates of the pure dioxane-degrading culture (Isolate A) were amplified using PCR and analyzed on DGGE. *Lane 1* Colony A, *Lanes 2 and 3* Isolate A, *Lane 4* Colony B, *Lane 5* the enriched consortium, and *Lane 6* Colony C

The results in Fig. 5 were obtained when whole cells were used as the PCR template and had the same outcome as when extracted DNA was used (data not shown).

Identification of the dioxane degrader

The major 16S rDNA PCR product of the dioxane-degrading microorganism was excised from DGGE gels, eluted, re-amplified, and sequenced. It exhibited 99% homology with *Flavobacterium* sp. IP10, and *cf. Cytophaga* sp. MDA2507 using NCBI BLAST. *Flavobacterium* is the type genus in the family *Flavobacteriaceae* dealing with *Flavobacterium* and *Cytophage*-like bacteria (Bernardet and Bowman 2006) and its classification was described by Bernardet et al. (2002). Since *cf. Cytophaga* sp. is unclassified (NCBI taxonomy database) and has a high 16S rDNA similarity to *Flavobacterium*, the enriched dioxane degrader can be tentatively classified as a *Flavobacterium*. Further phylogenetic analysis showed that the dioxane degrader in this study is distinct from all previously reported dioxane and THF degraders (Fig. 6).

Effect of THF concentration on dioxane degradation by the purified dioxane-degrader

To minimize using THF as a primary carbon source, an inoculum of the dioxane-degrading *Flavobacterium* was prepared on glucose and used to evaluate the effect of THF concentration on dioxane degradation. The rate and extent of dioxane degradation increased with increasing THF concentration (Fig. 7).

Discussion

Bioremediation is an attractive method to remove dioxane from the environment. However, few

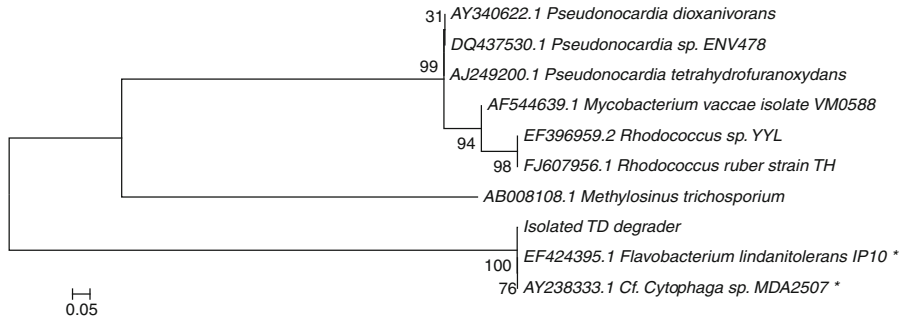

Fig. 6 Dendrogram based on the polygenetic analysis of 16S rDNA showing the relationship of the isolated dioxane degrader in this paper with previously reported dioxane and THF degraders. Since the 16S sequence of *Rhodococcus ruber* 219 and *Mycobacterium vaccae* JOB 5 are not available in GenBank, *Rhodococcus ruber* strain TH and *Mycobacterium vaccae* isolate VM0588 were used in the phylogenetic analysis. (*) *Flavobacterium lindanitolerans* strain IP10 and *Cf. Cytophaga* sp. MDA2507 are not reported dioxane or THD degraders but were used as representative of the best BLAST hit in GenBank. The bootstrap values were based on 1000 resamplings

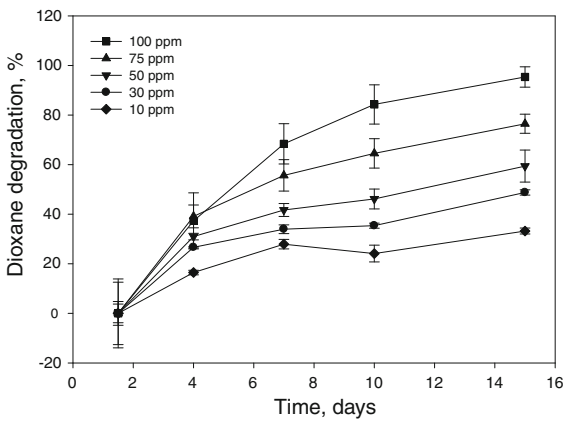

Fig. 7 Effect of THF concentration on dioxane degradation. The Isolate A (a purified dioxane degrader from Colony A) was cultured in BSM medium with 10 g/l glucose and then inoculated into fresh BSM medium containing 100 ppm dioxane and 10 (*filled diamond*), 30 (*filled circle*), 50 (*filled down-pointing triangle*), 75 (*filled up-pointing triangle*), or 100 (*filled square*) ppm of THF. Each *point* represents the average of two replicate cultures and the *error bars* represent the range of values obtained

microorganisms have been reported to degrade it. In this study, a *Flavobacterium* was enriched from dioxane-contaminated soil. The enriched culture did not use dioxane as the sole source of carbon and energy, but co-metabolically degraded it in the presence of THF. To the authors' knowledge, biodegradation of dioxane by *Flavobacterium* has not been reported to date. *Flavobacterium* is taxonomically distinct from previously identified dioxane-degrading bacteria. *Pseudonocardia dioxanivorans* CB 1190, *Pseudonocardia* K1, and *Pseudonocardia* sp. ENV 478, are in the genus *Pseudonocardia*, and *Mycobacterium vaccae* JOB 5, and *Rhodococcus rubber* 219 are in the Suborder of *Corynebacterineae*, and are all in the Order of *Actinomycetales*. *Flavobacterium* is in a different Phylum. This distinction has also been substantiated by phylogenetic and distance analyses (Fig. 6) (Yao et al. 2009).

Mahendra and Alvarez-Cohen (2006) have shown that microorganisms which express monooxygenases can degrade dioxane, and such a degradation pathway has been proposed by Vainberg et al. (2006). Based on bioinformatic approaches and the NCBI protein database, monooxygenases exist in *Flavobacterium* species. Although this information indicates that an oxygenase may be involved in dioxane degradation in *Flavobacterium*, naphthalene monooxygenase activity was not detected in our culture using the method of Brusseau et al. (1990). Therefore, a different degradation mechanism may be utilized in *Flavobacterium*.

Plating microorganisms on nutrient rich agar is a standard technique to obtain or isolate contaminant degraders from environmental sources. However, in a similar dioxane enrichment experiment, attempts to isolate dioxane degraders on tryptic soy agar plates were unsuccessful (Zenker et al. 2000). The authors postulated a number of reasons for such a negative outcome: (1) their culture might have lost its degrading ability upon growth on rich media; or (2) the colonies were too small to be observed or handled

because degraders grow slowly on nutrient plates; or (3) the syntrophic association with other microorganisms did not allow the degrader to form colonies. These difficulties were also observed early in our study. We were unable to isolate dioxane degraders when we initially transferred the enriched culture onto nutrient rich agar plates. To assess why it may be challenging to isolate dioxane degraders on nutrient plates, PCR and DGGE were used to analyze the enriched consortium and the cultures from the enrichment process. Colonies B and C did not degrade dioxane because they were different from the dioxane-degrading microorganism, and not because they had lost the ability to degrade dioxane when cultured on a rich medium. Although *Flavobacterium* was the dominant organism in the enriched consortium, the non-dioxane degraders (Colonies B and C) grew more quickly on the glucose-supplemented agar plates than Colony A (which was predominantly *Flavobacterium*), indicating that the major problem underlying the recovery of the dioxane degrader on nutrient rich agar was its slower growth on glucose. This might be a common problem in the isolation of an axenic culture or a stable consortium with degradation capabilities since contaminant degraders are usually enriched from groundwater, soil or sludge, from a diverse range of microbes. The additional molecular techniques employed in this study proved to be useful assessment tools that complemented the traditional microbial isolation techniques used for establishing pure cultures of contaminant degraders. Whole cells as the PCR template has been previously successful with a pure culture (Fode-Vaughan et al. 2003) or when diversity is quite limited (Klocke and Mundt 2004) as in this case.

To biodegrade dioxane at industrial-scale levels, it would be crucial to possess the ability to prepare the degrading cultures in large quantities. Most dioxane-degrading cultures use either dioxane or THF as the source of carbon and energy. Since both chemicals are toxic and microbial growth rates in dioxane/THF are usually slow, culture maintenance and efficient production of large numbers of cells become a challenge. Maintaining a dioxane degrader on a carbon source structurally dissimilar to dioxane without losing the ability to degrade dioxane was previously unsuccessful (Parales et al. 1994; Zenker et al. 2000). While the carbon sources of the propanotroph SL-D (Fam et al. 2005), and *M. trichosporium* OB3b (Mahendra and Alvarez-Cohen 2006) are less toxic, both propane and methane are flammable and must be treated with caution. The low solubility of propane and methane will result in mass transfer limitation and lead to a low cell density. The *Flavobacterium* reported in this study was able to grow on glucose, and have a stable dioxane degradation capability after several transfers between glucose and THF/dioxane. The ability to grow on glucose without losing dioxane-degrading capabilities is highly advantageous. The use of glucose is more cost-effective and safer than THF or dioxane and is thus more beneficial in large-scale industrial applications. The *Flavobacterium* grows more quickly on glucose than on THF and dioxane, allowing faster production of high density cell cultures suitable for industrial-scale applications.

Since *Flavobacterium* can be grown and maintained on glucose without losing its dioxane-degrading capability, a bioremediation strategy can be developed for industrial-scale applications. Cultures can be grown first on glucose to obtain high cell densities before adding THF to stimulate dioxane degradation. After THF concentration reaches a low level through degradation, the activated *Flavobacterium* can then be applied in a dioxane bioremediation process such as for *ex situ* treatment of dioxane-contaminated groundwater in a bioreactor or an in situ bioaugmentation treatment.

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