Biodegradation (2016) 27:277–286
DOI 10.1007/s10532-016-9772-7

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ORIGINAL PAPER

# 1,4-Dioxane degradation potential of members of the genera *Pseudonocardia* and *Rhodococcus*

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Received: 17 May 2016 / Accepted: 7 September 2016 / Published online: 13 September 2016
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**Abstract** In recent years, several strains capable of degrading 1,4-dioxane have been isolated from the genera *Pseudonocardia* and *Rhodococcus*. This study was conducted to evaluate the 1,4-dioxane degradation potential of phylogenetically diverse strains in these genera. The abilities to degrade 1,4-dioxane as a sole carbon and energy source and co-metabolically with tetrahydrofuran (THF) were evaluated for 13 *Pseudonocardia* and 12 *Rhodococcus* species. *Pseudonocardia dioxanivorans* JCM 13855$^T$, which is a 1,4-dioxane degrading bacterium also known as *P. dioxanivorans* CB1190, and *Rhodococcus aetherivorans* JCM 14343$^T$ could degrade 1,4-dioxane as the sole carbon and energy source. In addition to these two strains, ten *Pseudonocardia* strains could degrade THF, but no *Rhodococcus* strains could degrade THF. Of the ten *Pseudonocardia* strains, *Pseudonocardia acacia* JCM 16707$^T$ and *Pseudonocardia asaccharolytica* JCM 10410$^T$ degraded 1,4-dioxane co-metabolically with THF. These results indicated that 1,4-dioxane degradation potential, including degradation for growth and by co-metabolism with THF, is possessed by selected strains of *Pseudonocardia* and *Rhodococcus*, although THF degradation potential appeared to be widely distributed in *Pseudonocardia*. Analysis of soluble di-iron monooxygenase (SDIMO) α-subunit genes in THF and/or 1,4-dioxane degrading strains revealed that not only THF and 1,4-dioxane monooxygenases but also propane monooxygenase-like SDIMOs can be involved in 1,4-dioxane degradation.

**Keywords** 1,4-dioxane degradation · *Pseudonocardia* · *Rhodococcus* · Soluble di-iron monooxygenase gene

**Electronic supplementary material** The online version of this article (doi:10.1007/s10532-016-9772-7) contains supplementary material, which is available to authorized users.

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## Introduction

1,4-Dioxane is a cyclic ether which is an industrially important solvent used in paints, lacquers, cosmetics, deodorants, fumigants and detergents. It is also formed

as a by-product during the manufacture of polyesters. 1,4-Dioxane has high water solubility, low volatility, low adsorbability to solids, and low susceptibility to chemical and biological reactions (Wolfe and Jeffers 2000; Agency for Toxic Substances and Disease Registry (ATSDR) 2012; Sei et al. 2013a; Stepien et al. 2014). Because of these properties, once 1,4-dioxane is released into water environments, it can persist for a long period.

1,4-Dioxane contamination has been extensively detected in surface water, groundwater, and landfill leachate throughout the world (Lesage et al. 1990; Abe 1999; Isaacson et al. 2006; Fujiwara et al. 2008; Agency for Toxic Substances and Disease Registry (ATSDR) 2012; Chiang et al. 2012; Sei et al. 2013a; Stepien et al. 2014). The major causes of the water contamination are illegal dumping of industrial wastes, and incomplete treatment and leakage of landfill leachate and industrial wastewater. Because 1,4-dioxane is a group 2B (possible) human carcinogen (International Agency for Research on Cancer (IARC) 1999), appropriate cleanup of 1,4-dioxane-contaminated water is an important public issue. However, most of the conventional physical and chemical remediation methods are not effective to decontaminate 1,4-dioxane (Adams et al. 1994). Although advanced oxidation processes (AOPs) such as the combination of ozone and hydrogen peroxide treatments can efficiently decompose 1,4-dioxane (Adams et al. 1994; Kim et al. 2006; Kishimoto et al. 2008), their application for the cleanup of a 1,4-dioxane-contaminated environment would be unrealistic in light of cost- and energy-effectiveness. Consequently, the development of low cost, energy efficient and environmentally friendly alternatives is strongly desired.

Bioremediation is a promising remediation technique because of its cost effectiveness, inherent eco-friendly properties, and the potential for complete decomposition of harmful compounds. Although 1,4-dioxane had been recognized as a recalcitrant to biodegradation, bacterial strains capable of degrading 1,4-dioxane as the sole carbon and energy source or co-metabolically with tetrahydrofuran (THF), methane, propane, and toluene have been isolated and characterized during the last two decades (Table S1 in Online Resource 1). Furthermore, several recent studies have confirmed the intrinsic 1,4-dioxane biodegradation potential of contaminated environments by microcosm studies (Li et al. 2010; Sei et al. 2010; Li et al. 2014; 2015) or field surveys (Chiang et al. 2012). These findings suggested the feasibility of in situ bioremediation for 1,4-dioxane contaminated environments. However, little is known concerning 1,4-dioxane degrading microorganisms compared with what is known about strains that can degrade petroleum hydrocarbons like benzene, toluene, ethylbenzene, and xylene (Alvarez and Vogel 1991; Cao et al. 2009; Weelink et al. 2010; Sun and Cupples 2012), and chlorinated solvents like trichloroethylene (Damborský 1999; Shukla et al. 2014) for which in situ bioremediation technologies have been well established. To establish effective bioremediation strategies for 1,4-dioxane contamination, further knowledge of 1,4-dioxane degrading microorganisms is necessary.

Although recent research revealed that a relatively wide range of bacterial species possess the ability to degrade 1,4-dioxane, the majority are nocardioform actinomycetes, belonging to genera such as *Pseudonocardia* (Parales et al. 1994; Kohlweyer et al. 2000; Kämpfer and Kroppenstedt 2004; Prabahar et al. 2004; Vainberg et al. 2006; Sei et al. 2013a; Matsui et al. 2016) and *Rhodococcus* (Bernhardt and Diekmann 1991; Deeb and Alvarez-Cohen 1999; Sei et al. 2013b) (Table S1 in Online Resource 1). Thus, an understanding of the 1,4-dioxane degrading potential and properties of bacterial species of these genera is important for developing bioremediation technologies for 1,4-dioxane contamination. Therefore, this study was conducted to evaluate the 1,4-dioxane degradation potential of phylogenetically diverse members of *Pseudonocardia* and *Rhodococcus*. The ability of the test strains to degrade 1,4-dioxane as the sole carbon and energy source and co-metabolically degrade 1,4-dioxane in the presence of THF was evaluated. The genes encoding soluble di-iron monooxygenase (SDIMO), which is known to be associated with the initial oxidation of 1,4-dioxane (Li et al. 2013), in the test strains were also analyzed.

## Materials and methods

### Test strains

A total of 13 and 12 phylogenetically diverse species of *Pseudonocardia* and *Rhodococcus*, respectively,

were selected to evaluate their 1,4-dioxane degradation potential (Figs. S1 and S2 in Online Resource 1). Type strains for the 25 species (Table 1) were provided by the RIKEN BRC through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, and were used as test strains in this study. *Pseudonocardia dioxanivorans* JCM 13855$^{T}$, which is known as *P. dioxanivorans* CB1190 (Parales et al. 1994), was used as the positive control as it was capable of degrading 1,4-dioxane as a sole carbon and energy source.

## Culture media

MGY medium (malt extract 10 g/L, D(+)-glucose 4 g/L, yeast extract 4 g/L, pH 7.3) and ISP medium 2 (Becton, Dickinson and Company, Sparks, MD, USA) were used as liquid and solid media, respectively, for routine cultivation of test strains. Basal salt medium (BSM) (Parales et al. 1994) adjusted to pH 7.0 was used for 1,4-dioxane degradation experiments.

## 1,4-Dioxane degradation experiments

Prior to the 1,4-dioxane degradation experiments, test strains were precultivated in MGY medium at 28 °C, except for *Pseudonocardia acaciae* JCM 16707$^{T}$ and *Pseudonocardia thermophila* JCM 3095$^{T}$, which were precultivated at 37 °C. The cells of the precultivated strains were harvested by centrifugation (8500×*g*, 4 °C, 5 min) and washed twice with sterilized 0.85 % (w/v) NaCl. Then, the washed cells were inoculated in 50-ml glass vials containing 20 ml of BSM to give a final cell density (determined by optical density at 600 nm ($OD_{600}$)) of 1.0. 1,4-Dioxane was added to BSM at 20 mg/L as the sole carbon and energy source for 1,4-dioxane utilization experiments. 1,4-Dioxane and THF were added at 20 and 50 mg/L, respectively, for the 1,4-dioxane co-metabolic degradation experiments. Control systems without bacterial inoculation were also prepared for each experiment. The cultures were incubated at 28 °C with rotary shaking at 150 rpm, while *P. acaciae* JCM 16707$^{T}$ and *P. thermophila* JCM 3095$^{T}$ were incubated at 37 °C, excepting for the screening experiments to select THF degrading strains in the 1,4-dioxane co-metabolic degradation experiments. 1,4-Dioxane utilization experiments were conducted for 14 days. For the 1,4-dioxane co-metabolic degradation experiments, first, screening experiments to select the THF degrading strains were conducted for 7 days, and then experiments to examine the co-metabolic degradation of 1,4-dioxane with THF were carried out for 14 days on the selected strains. Aliquots (0.5 ml) were periodically collected, centrifuged (20,000×*g*, 4 °C, 5 min), filtered through a cellulose acetate filter (pore size 0.45 µm, Advantec, Tokyo, Japan), and subjected to 1,4-dioxane and THF quantification. All of the degradation experiments were conducted in duplicate. Statistical significance for THF and 1,4-dioxane degradation by test strains was determined by Student's *t* test with $p < 0.05$ using the Prism 6J for Windows program (GraphPad Software, La Jolla, CA, USA).

## Chemical analysis

Bacterial cell density ($OD_{600}$) was determined with a PD-3000UV spectrophotometer (Apel, Saitama, Japan) or a UVmini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). The concentrations of 1,4-dioxane and THF were determined using a gas chromatograph GC2014 (Shimadzu) equipped with a flame-ionization detector (FID) and a 2.1-m (3.2-mm i.d.) glass column packed with Gaskuropack 54 (GL Science, Tokyo, Japan). Nitrogen gas was applied as the carrier gas at a flow rate of 55 ml/min. The injector and column oven temperature was set at 200 °C, while the detector temperature was at 230 °C. The injection volume of the filtered samples was 5 µl, and the detection limit of 1,4-dioxane and THF was 1 mg/L.

## Analysis of soluble di-iron monooxygenase genes

1,4-Dioxane degradation enzymes are included in the large SDIMO family, which includes multicomponent enzymes that catalyze the initial oxidation of a variety of hydrocarbons (Coleman et al. 2006; Li et al. 2013). The presence of SDIMO genes in test strains was examined by PCR using the NVC57 and NVC66 primer sets, which was specifically designed to detect the conserved region in the SDIMO α-subunit genes (Coleman et al. 2006). *Pseudonocardia* sp. D17 [a 1,4-dioxane utilizing bacterium (Sei et al. 2013a)] and *Rhodococcus ruber* T1 and T5 [THF degrading bacteria capable of co-metabolically degrading 1,4-

**Table 1** Results from the 1,4-dioxane degradation experiments

| Genus | Test strain | 1,4-Dioxane utilization experiment | 1,4-Dioxane co-metabolic degradation experiment | |
|---|---|---|---|---|
| | | 1,4-Dioxane degradation | THF degradation | 1,4-Dioxane co-metabolic degradation |
| *Pseudonocardia* | *P. acaciae* JCM 16707$^T$ | – | + | + |
| | *P. ammonioxydans* JCM 12462$^T$ | – | – | – |
| | *P. asaccharolytica* JCM 10410$^T$ | – | + | + |
| | *P. autotrophica* JCM 4348$^T$ | – | + | – |
| | *P. carboxydivorans* JCM 14827$^T$ | – | + | – |
| | *P. chloroethenivorans* JCM 12679$^T$ | – | – | – |
| | *P. dioxanivorans* JCM 13855$^T$ | + | + | + |
| | *P. halophobica* JCM 9421$^T$ | – | + | – |
| | *P. hydrocarbonoxydans* JCM 3392$^T$ | – | + | – |
| | *P. petroleophila* JCM 3378$^T$ | – | + | – |
| | *P. sulfidoxydans* JCM 10411$^T$ | – | + | – |
| | *P. thermophila* JCM 3095$^T$ | – | + | – |
| | *P. yunnanensis* JCM 9330$^T$ | – | + | – |
| *Rhodococcus* | *R. aetherivorans* JCM 14343$^T$ | + | + | + |
| | *R. chlorophenolicus* JCM 7439$^T$ | – | – | – |
| | *R. corallinus* JCM 3199$^T$ | – | – | – |
| | *R. corynebacterioides* JCM 3376$^T$ | – | – | – |
| | *R. equi* JCM 1311$^T$ | – | – | – |
| | *R. erythropolis* JCM 3201$^T$ | – | – | – |
| | *R. gordoniae* JCM 12658$^T$ | – | – | – |
| | *R. opacus* JCM 9703$^T$ | – | – | – |
| | *R. pyridinivorans* JCM 10940$^T$ | – | – | – |
| | *R. rhodochrous* JCM 3202$^T$ | – | – | – |
| | *R. ruber* JCM 3205$^T$ | – | – | – |
| | *R. zopfii* JCM 9919$^T$ | – | – | – |

+ Capable of degrading 1,4-dioxane/THF within experimental periods; – cannot degrade 1,4-dioxane/THF within experimental periods

dioxane (Sei et al. 2013b)] were also analyzed for their SDIMO genes. PCR was conducted using the following thermal profile: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 1 min, and final extension at 72 °C for 5 min. This protocol gives PCR products of approximately 420 bp. The PCR products were analyzed by electrophoresis on a 1.5 % (w/v) agarose gel stained with SYBR Green I (Takara Bio, Shiga, Japan), after which they were purified with NucleoSpin Gel and PCR Clean-up (Macherey–Nagel, Düren, Germany) and sequenced on an Applied Biosystems 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA) with primers NVC57 and NVC66 at Macrogen Janan (Tokyo, Japan). The nucleotide sequences obtained from the sequencing with both primers were combined to yield single sequences. The nucleotide sequences determined for the test strains were compared with those in the NCBI database using the BLAST search program (http://www.ncbi.nlm.nih.gov/blast/). Then, the nucleotide sequences of the test strains in this study and reference strains in the NCBI database were aligned using CLUSTAL W (Eddy 1995), and a phylogenetic tree was produced using TreeView X (Page 1996).

### Nucleotide sequence accession numbers

The partial sequences of the SDIMO α-subunit genes determined in this study were deposited in the DDBJ/EMBL/GenBank databases under accession numbers LC114132 to LC114146.

## Results

### 1,4-Dioxane utilization ability

In the 1,4-dioxane utilization experiments, where 1,4-dioxane was added as the sole carbon and energy source, 1,4-dioxane was significantly degraded by *P. dioxanivorans* JCM 13855$^T$ and *Rhodococcus aetherivorans* JCM 14343$^T$ ($p < 0.05$), while 1,4-dioxane was not significantly degraded by the other 23 test strains within 14 days (Table 1). *P. dioxanivorans* JCM 13855$^T$ completely degraded 20 mg/L of 1,4-dioxane within 4 days after a 2 days lag period (Fig. 1a), which agreed with the previous finding that this strain has inducible 1,4-dioxane degradation enzymes (Kelly et al. 2001). *R. aetherivorans* JCM 14343$^T$ degraded 20 mg/L of 1,4-dioxane completely within 1 day after a 9 h lag period (Fig. 1b).

### 1,4-Dioxane co-metabolic degradation ability with THF

Prior to the evaluation of the ability of the test strains to degrade 1,4-dioxane co-metabolically with THF, we first screened their THF degradation potential. Among the 13 *Pseudonocardia* strains, 11 strains except for *Pseudonocardia ammonioxydans* JCM 12462$^T$ and *Pseudonocardia chloroethenivorans* JCM 12679$^T$ were capable of significantly degrading THF within 7 days (Table 1). Among the 12 *Rhodococcus* strains, THF was significantly degraded within 7 days by *R. aetherivorans* JCM 14343$^T$, while THF degradation did not occur within 7 days by the other 11 test strains.

The 12 THF degrading strains were then examined for their 1,4-dioxane co-metabolic degradation ability with THF for 14 days. In addition to *P. dioxanivorans* JCM 13855$^T$ and *R. aetherivorans* 14343$^T$, both of which were capable of utilizing 1,4-dioxane for their growth, *P. acaciae* JCM 16707$^T$ and *Pseudonocardia asaccharolytica* JCM 10410$^T$ could significantly degrade 1,4-dioxane subsequent to THF degradation (Table 1). *P. acaciae* JCM 16707$^T$ completely degraded THF within 12 days without a lag period, and also completely degraded 1,4-dioxane within 14 days after a lag period of 10 days (Fig. 2a). *P. asaccharolytica* JCM 10410$^T$ degraded nearly 80 % of the initial THF within 14 days without a lag period (Fig. 2b). 1,4-Dioxane degradation by this strain was initiated after a lag period of 12 days, and nearly 45 % of the initial 1,4-dioxane was degraded after 14 days. Co-metabolic degradation of 1,4-dioxane with THF did not occur within 14 days by the other eight strains (Table 1; Fig. 2c, d, and Fig. S3 in Online Resource 1).

### Possession of SDIMO genes

The presence of SDIMO genes in the 12 strains capable of degrading THF and/or 1,4-dioxane, and

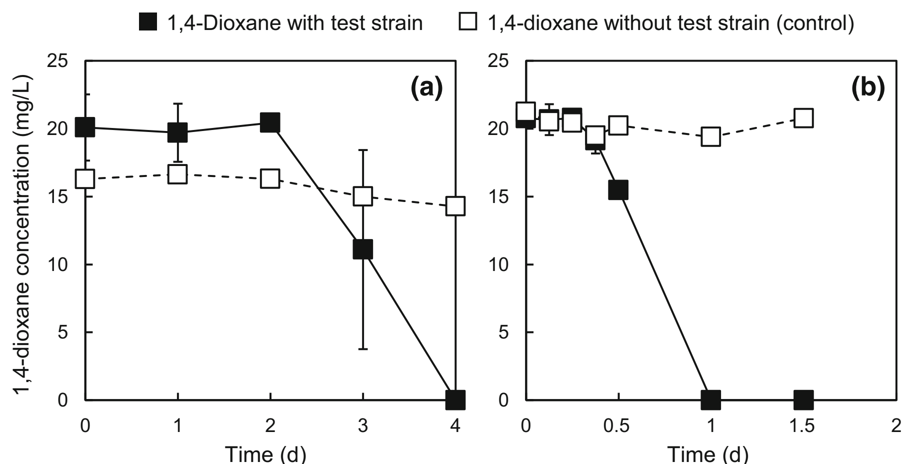


**Fig. 1** 1,4-Dioxane degradation by *Pseudonocardia dioxanivorans* JCM 13855$^T$ (**a**) and *Rhodococcus aetherivorans* JCM 14343$^T$ (**b**) as the sole carbon and energy source. *Error bars* indicate the data range in duplicate experiments

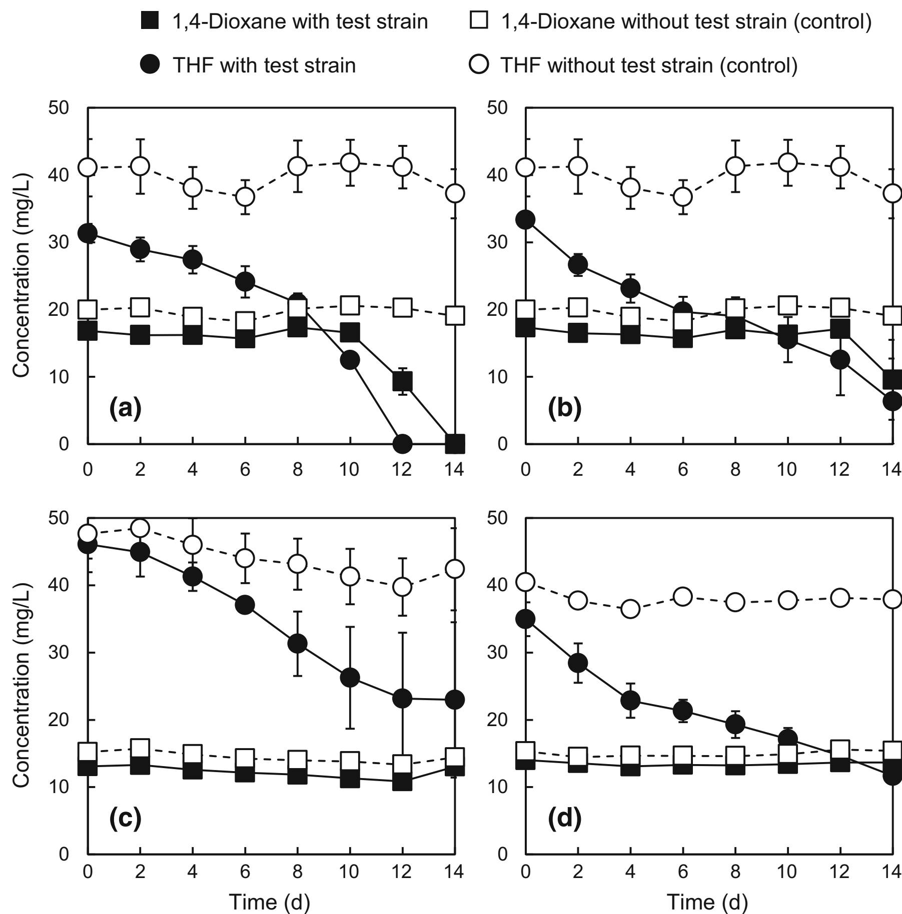


**Fig. 2** Co-metabolic degradation of 1,4-dioxane with tetrahydrofuran (THF) by *Pseudonocardia acaciae* JCM 16707$^T$ (**a**) and *Pseudonocardia asaccharolytica* JCM 10410$^T$ (**b**). Examples of THF degradation without co-metabolic degradation of 1,4-dioxane by *Pseudonocardia hydrocarbonoxydans* JCM 3392$^T$ (**c**) and *Pseudonocardia thermophila* JCM 3095$^T$ (**d**) are also shown. *Error bars* indicate the data range in duplicate experiments

three recently isolated 1,4-dioxane degrading strains (*Pseudonocardia* sp. D17, *R. ruber* T1 and *R. ruber* T5) were examined by a PCR assay specific to the SDIMO α-subunit genes. PCR products with anticipated size were obtained from all of the strains. The phylogenetic tree constructed based on the nucleotide sequences of the PCR products from the 15 strains determined in this study and those of known SDIMO α-subunit genes is shown in Fig. 3. From the nucleotide sequence of the SDIMO α-subunit gene of the control strain *P. dioxanivorans* JCM 13855$^T$ (*P. dioxanivorans* CB1190), the presence of a putative 1,4-dioxane monooxygenase α-subunit gene (*dxmA* gene) was confirmed. The nucleotide sequences of the SDIMO α-subunit genes of *Pseudonocardia* sp. D17, *R. ruber* T1, and *R. ruber* T5 were 100 % identical to that of the THF monooxygenase α-subunit gene (*thmA*) of *Rhodococcus* sp. YYL.

The nucleotide sequences of the SDIMO α-subunit genes of the other 11 strains (i.e., *R. aetherivorans* JCM 14343$^T$ and 10 *Pseudonocardia* strains with THF degradation ability) were closely related to the sequences of putative propane monooxygenase hydroxylase large subunit genes (*prmA*-like genes) of previously characterized strains (Fig. 3). The SDIMO α-subunit gene of *R. aetherivorans* JCM 14343$^T$ was identical to the *prmA* gene of *Rhodococcus* sp. RR1, and allocated into a *prmA* gene subcluster for *Rhodococcus* strains. It was highly different from the SDIMO α-subunit genes classified into the *thm*/*dxm* gene cluster (similarity: 58.4–58.6 %). The SDIMO α-subunit genes of the 10 *Pseudonocardia*

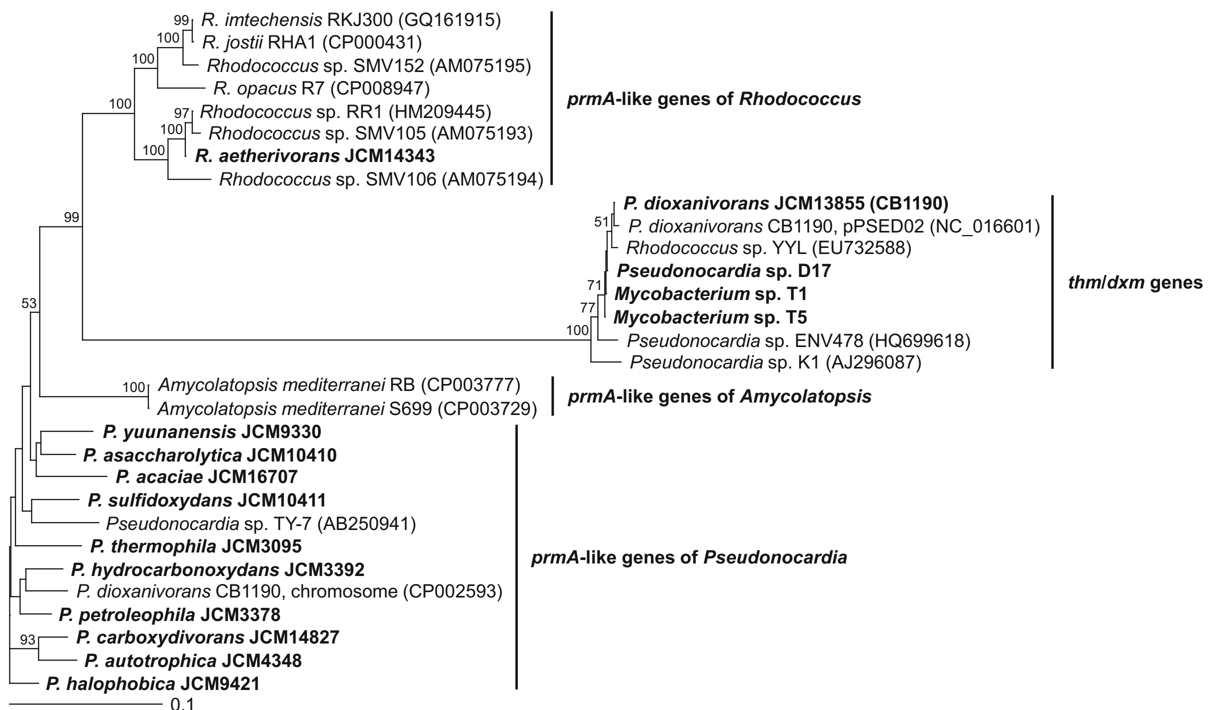


**Fig. 3** Phylogenetic tree constructed based on the nucleotide sequences of soluble di-iron monooxygenase α-subunit genes of the test strains and the ones previously reported for cultured strains. Sequences for the strains indicated in boldface were determined in this study. Numbers adjacent to the branches indicate the bootstrap values based on 1000 replicates. *Bar* indicates 0.1 substitutions per sequence position

strains had 89.2–95.6 % similarity to each other. Of these, that of *P. acaciae* JCM 16707$^T$ had the highest similarity (91.0 %) to the *prm1A* gene of *Pseudonocardia* sp. TY-7, while those of the other nine strains had the highest similarity of 92.0–95.9 % to the *prmA*-like gene located on the chromosome of *P. dioxanivorans* CB1190.

## Discussion

Several strains of the genus *Pseudonocardia* are capable of degrading 1,4-dioxane for growth (Parales et al. 1994; Kämpfer and Kroppenstedt 2004; Sei et al. 2013a; Matsui et al. 2016), or co-metabolically (Kohlweyer et al. 2000; Vainberg et al. 2006). In this study, although 1,4-dioxane degradation as the sole carbon and energy source by *P. dioxanivorans* JCM 13855$^T$ (also known as *P. dioxanivorans* CB1190 (Parales et al. 1994)) was confirmed, none of the other 12 *Pseudonocardia* strains could degrade 1,4-dioxane as the sole carbon and energy source (Table 1). It was surprising that *Pseudonocardia carboxydivorans* JCM 14827$^T$ was not capable of degrading 1,4-dioxane for its growth or by co-metabolism with THF, although its 16S rRNA gene was 100 % homologous to *Pseudonocardia* sp. RM-31, a strain that was very recently isolated as a novel 1,4-dioxane assimilating bacterium (Matsui et al. 2016). Different 1,4-dioxane degradation abilities in the two *P. carboxydivorans* strains suggest that their 1,4-dioxane assimilation ability is strain-specific rather than species-specific. By contrast, 10 of the 12 *Pseudonocardia* strains other than *P. dioxanivorans* JCM 13855$^T$ were capable of degrading THF, and two of them (*P. acaciae* JCM 16707$^T$ and *P. asaccharolytica* JCM 10410$^T$) enabled co-metabolic 1,4-dioxane degradation with THF. To our knowledge, this is the first study to report the THF degradation and co-metabolic 1,4-dioxane degradation potential of these *Pseudonocardia* strains. Taken together, the results of this study indicated that limited *Pseudonocardia* strains possess the ability to degrade 1,4-dioxane degradation as the sole carbon and energy source. Additionally, our results indicated that

phylogenetically diverse *Pseudonocardia* species/strains commonly possess the potential to degrade THF for growth and some can co-metabolically degrade 1,4-dioxane with THF.

Within the genus *Rhodococcus*, 1,4-dioxane degradation as the sole carbon and energy source was first reported for *R. ruber* 219 (Bernhardt and Diekmann 1991). It was recently reported that *R. ruber* T1 and T5 can co-metabolically degrade 1,4-dioxane with THF (Sei et al. 2013b). However, *R. ruber* JCM $3205^T$ examined in this study could not degrade either 1,4-dioxane or THF. This suggested that 1,4-dioxane and THF degradation ability is a strain-specific property in *R. ruber*. By contrast, this study found that *R. aetherivorans* JCM $14343^T$ could degrade 1,4-dioxane as the sole carbon and energy source. Also, we have confirmed in another study that the strain can utilize 1,4-dioxane for its growth, with the cell yield of 0.031 mg-protein/mg-1,4-dioxane (unpublished data). *R. aetherivorans* JCM $14343^T$ (originally named strain $10bc312^T$) was isolated from an enrichment obtained from the petrochemical biotreater sludge of a chemical effluent treatment plant as a methyl *tert*-butyl ether degrading strain (Goodfellow et al. 2004), but its degradation ability for cyclic ethers has not been reported. Although another strain of *R. aetherivorans* was reported to degrade THF (Tajima et al. 2012), toluene (Hori et al. 2009), and a spectrum of petroleum compounds (Auffret et al. 2009), this is the first study to clarify that a *R. aetherivorans* strain can degrade 1,4-dioxane as the sole carbon and energy source. Additionally, the evidence from previous studies and this study indicates that *R. aetherivorans* may be a specific species in *Rhodococcus* that is capable of degrading both cyclic and non-cyclic recalcitrant ether compounds. It is likely that 1,4-dioxane degradation ability, including both utilization for growth and co-metabolic degradation with THF, is not distributed widely among the genus *Rhodococcus*, but is possessed by specific strains in limited species of the genus. Based on the phylogenetic composition of *Rhodococcus* (Fig. S2 in Online Resource 1), its 1,4-dioxane degradation ability is likely a specific function of some strains in a subcluster consisting of *R. aetherivorans* and *R. ruber*.

SDIMOs are multicomponent enzymes that catalyze the initial oxidation of a variety of hydrocarbons such as chlorinated solvents, aromatic hydrocarbons, alkanes and alkenes in phylogenetically and physiologically diverse bacteria (Coleman et al. 2006; Li et al. 2013). Previous studies have reported that THF and 1,4-dioxane monooxygenases (THM and DXM, respectively) are involved in 1,4-dioxane degradation for growth or by co-metabolism, and some propane monooxygenases (PMOs) can also co-metabolize 1,4-dioxane (Mahendra and Alvarez-Cohen 2006; Li et al. 2013). Nevertheless, our SDIMO gene analysis revealed that the SDIMO genes possessed by *R. aetherivorans* JCM $14343^T$, *P. acaciae* JCM $16707^T$ and *P. asaccharolytica* JCM $10410^T$, whose 1,4-dioxane degrading abilities were revealed in this study for the first time, were closely related to *prm* genes, and clearly separated from *thm*/*dxm* genes. This is the first study to identify the presence of PMO-like SDIMOs that are possibly involved in 1,4-dioxane degradation as the sole carbon and energy source (i.e., SDIMO of *R. aetherivorans* JCM $14343^T$). PMO-like SDIMOs are diverse in light of their 1,4-dioxane degradation abilities; some enable 1,4-dioxane degradation for the growth of host strains or by co-metabolism with primary substrates such as THF and propane, while the others cannot catalyze 1,4-dioxane degradation even by co-metabolism. This indicates the importance of not only THM/DXM-possessing microorganisms but also PMO-possessing microorganisms in the implementation of 1,4-dioxane bioremediation. Further genetic and enzymatic studies are needed to obtain a deeper understanding of the 1,4-dioxane degradation abilities of SDIMOs including THM/DXM and PMOs.

In conclusion, the 1,4-dioxane degradation potential of strains of *Pseudonocardia* and *Rhodococcus* was evaluated in this study. Our results revealed that 1,4-dioxane can be degraded by selected strains of *Pseudonocardia* and *Rhodococcus*, indicating that the 1,4-dioxane degradation potential by natural attenuation or biostimulation cannot by evaluated by the occurrence of those genera in 1,4-dioxane-contaminated sites. Another novel finding that PMO-like SDIMOs are possibly capable of degrading 1,4-dioxane not only by co-metabolism but also for growth suggests that the molecular tool previously developed based on THM/DXM genes (Li et al. 2014) would underestimate the 1,4-dioxane degradation potential of microbial communities in contaminated sites. Thus, a comprehensive molecular tool that can detect all of the SDIMO genes that enable 1,4-dioxane degradation is needed for adequate evaluation of the

1,4-dioxane degradation potential of microbial communities. Nevertheless, the evidence for the presence of phylogenetically diverse 1,4-dioxane degrading strains reported in previous studies (Table S1 in Online Resource 1) and this study would suggest that in situ bioremediation of 1,4-dioxane-contaminated water environments such as groundwater may be possible by dominance and/or selective activation of indigenous degraders through injecting appropriate growth and/or primary substrates even though the degraders may be minor constituents in the environment.

**Acknowledgments** This study was supported by Environment Research and Technology Development Fund (5B-1201) of the Ministry of the Environment, Japan, by the Adaptable and Seamless Technology Transfer Program through Target-driven R&D (A-STEP) of the Japan Science and Technology (JST), Japan, and by the Kurita Water and Environment Foundation (15A066).

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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