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Screening of fluorinated materials degrading microbes

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ABSTRACT

Isolation of bacterial strains capable of degrading fluorinated materials was described. 8 strains of *Actinobacteria* exhibited degradability of ethyl difluoroacetate (DFAc) was accumulated by bacteria, giving difluoroacetic acid and then fluoride ion. Further, 13 strains of *Actinobacteria* exhibited degradability of fluorobenzene and/or benzotrifluoride. In batch culture, growth of strains on fluorinated materials led to the release of fluoride ion.

1. Introduction

Fluorinated chemicals have had a marked impact on various fields such as pharmacology or functionalized materials which can lead to profound and unexpected results on biological activities and/or physical properties [1,2]. These biological and/or physical effects are due to the unique properties of fluorine such as a van der Waals radius (1.47 Å which is between that of hydrogen and oxygen), electronegativity (4.0 is the strongest one of all the atoms) and the high strength of the carbon–fluorine bond (C–F, 485 kJ/mol; C–H, 414 kJ/mol; C–OH, 359 kJ/mol; C–C, 347 kJ/mol; C–Cl, 339 kJ/mol), strongly contributing to the high stability of the fluorinated materials. While fluorinated materials are most stable compounds in the environment, nature seems to be reluctant to develop biological defluorination concerning *p*-fluoroaniline with horseradish peroxidase [3], fluoroacetate with monofluoroacetate dehalogenase (*Ipaeudomonas indoloxidans*, *P. cepacia*, *Moraxella* sp., *Burkholderia* sp.) [4,5] and biodegradability of trifluoroacetic acid [6]. Further, fluoraromotic compounds are biodegraded under aerobic conditions, although anaerobic degradation has also been reported [7]. However, in general, fluorinated chemicals are prominent xenobiotics and have low biodegradability due to their high stability. Consequently, these materials have received much less attention on the accumulation and biodegradation by bacteria, fungi and yeasts in the environment. On the basis of the greenery chemistry, it is of great importance to establish processes to retransform them into organic matter, which they were originality.

Recently, we have reported the isolation and purification of fluoroacid dehalogenase which transforms 2,2-difluoroethanol to difluoroacetic acid and then accumulates difluoroacetic acid [8].

For our continuous studies on the greenery chemistry, we would like to describe the screening *Actinobacteria* which can degrade some fluorinated materials in order to enable such processes in a biological way.

2. Results and discussion

2.1. Screening of bacteria for the degradation of fluorinated materials

The degradation of fluorinated material such as ethyl difluoroacetate (EDFA), fluorobenzene (FB) and/or benzotrifluoride (BTF) was carried out at 28 °C for 2 weeks aerobically in the test tube (see Section 4). Of 250 *Actinobacteria* checked, 8 bacteria could increase the fluoride ion by the decomposition of ethyl difluoroacetate as shown in Fig. 1. Concentration of fluoride ion was detected by ISE combination fluoride (ION pH/mV/ORP; Mettler-Toledo Group, Swiss). Bacteria (strain 064239) degraded EDFA up to 2%. Further, in the above manner, the degradation of FB or BTF was carried out at 28 °C for 2 weeks aerobically. After checking 350 *Actinobacteria*, 13 bacteria could increase the fluoride ion by the decomposition of FB or BTF. Bacteria (strain 065240) degraded fluorobenzene (up to 1.6%) and/or benzotrifluoride (up to 2.7%). A strain with the potential

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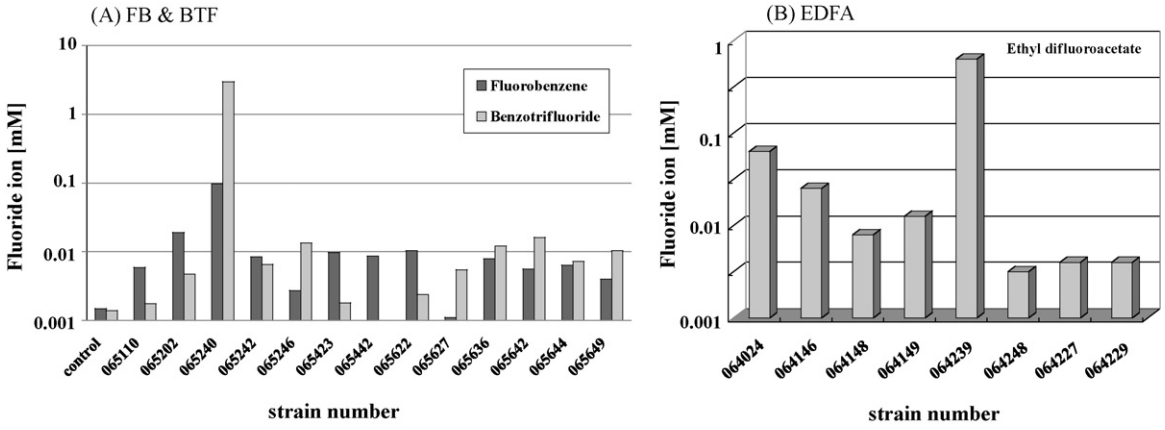

Fig. 1. Results of screening of C–F bond degradable microbes.

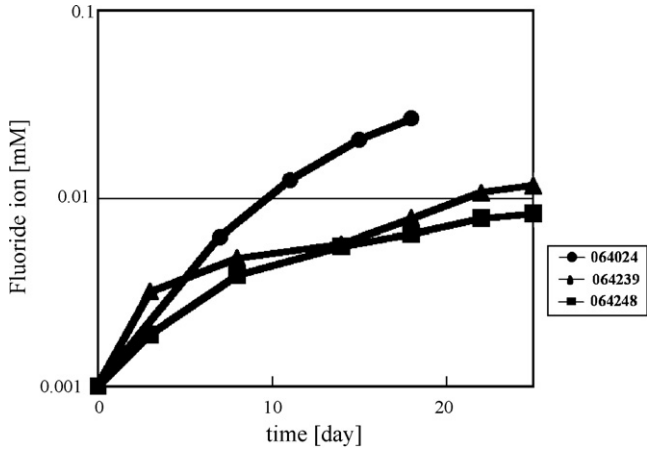

Fig. 2. Time course of biodegradation of ethyl difluoroacetate.

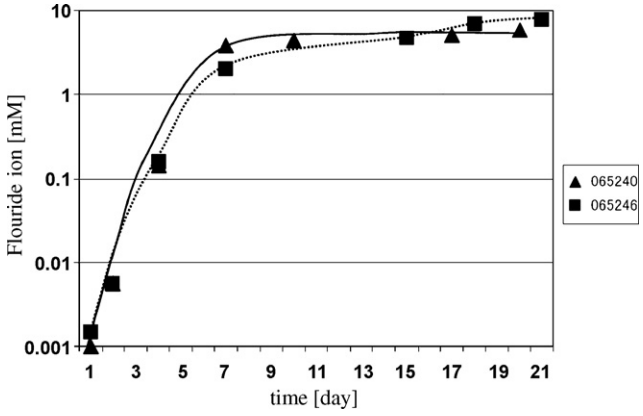

Fig. 4. Time course of biodegradation of benzotrifluride.

to rapidly degrade ethyl difluoroacetate, is isolated. In the cultivation, we have found that none of fluorinated material except difluoroacetic acid has been detected (see Section 4). From these results, we have found that difluoroacetic acid is isolated as an accumulating material from ethyl difluoroacetate. In the second stage, it seems that hydrolytic dehalogenation of difluoroacetic acid proceeded to produce the corresponding non-fluorinated material.

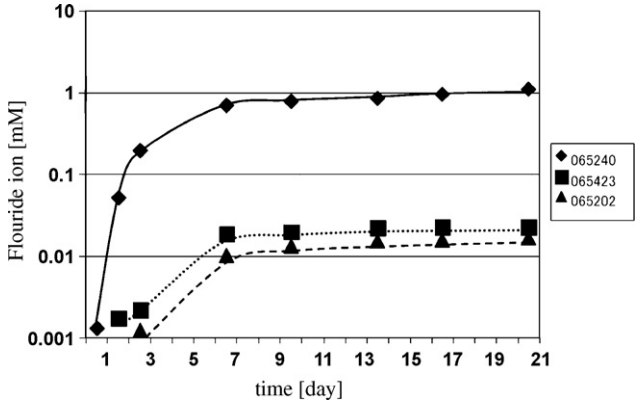

Fig. 3. Time course of biodegradation of fluorobenzene.

2.2. Time course of biodegradation

In the above cultivation, time course of biodegradation is shown in Figs. 2–4. In the cultivation shown in Fig. 2, we have found that difluoroacetic acid is isolated as an accumulating material from ethyl difluoroacetate. In contrast with Figs. 2 and 3, the time course shown in Fig. 4 suggests that the biodegradation of benzotrifluoride is smooth.

2.3. Identification

Strains were characterized and identified by the biochemical test based on Bergey's Manual of Determinative Bacteriology [9] and 16S rDNA sequences. The 16S rDNA of strains (064024,

Table 1
Primers.

Primer name	Sequence	Strain
9F	5′-GAGTTTGATCCTGGCTCAG	064024, 064248, 065240
1541R	5′-AAGGAGGTGATCCAGCC	064024, 064248, 065240
1510R	5′-GGCT ACCTTGTTACGA	064239
530F	5′-CAGGCTAGAGTGTGGTAG	064024, 064248
1015R	5′-CACGACACGAGCTGACG	064024, 064248
140-1	5′-ACCTCTTATCAGCAGGGACG	065240
140-2	5′-AAACTCAAAGGAATTGACGG	065240
140-r	5′-CGTGTTACTCACCCGTTCGC	065240
140-3′	5′-ACACATGCTACAATGGCCAG	065240
140R′	5′-AGCCATGCAGCCGAAGGTACG	065240

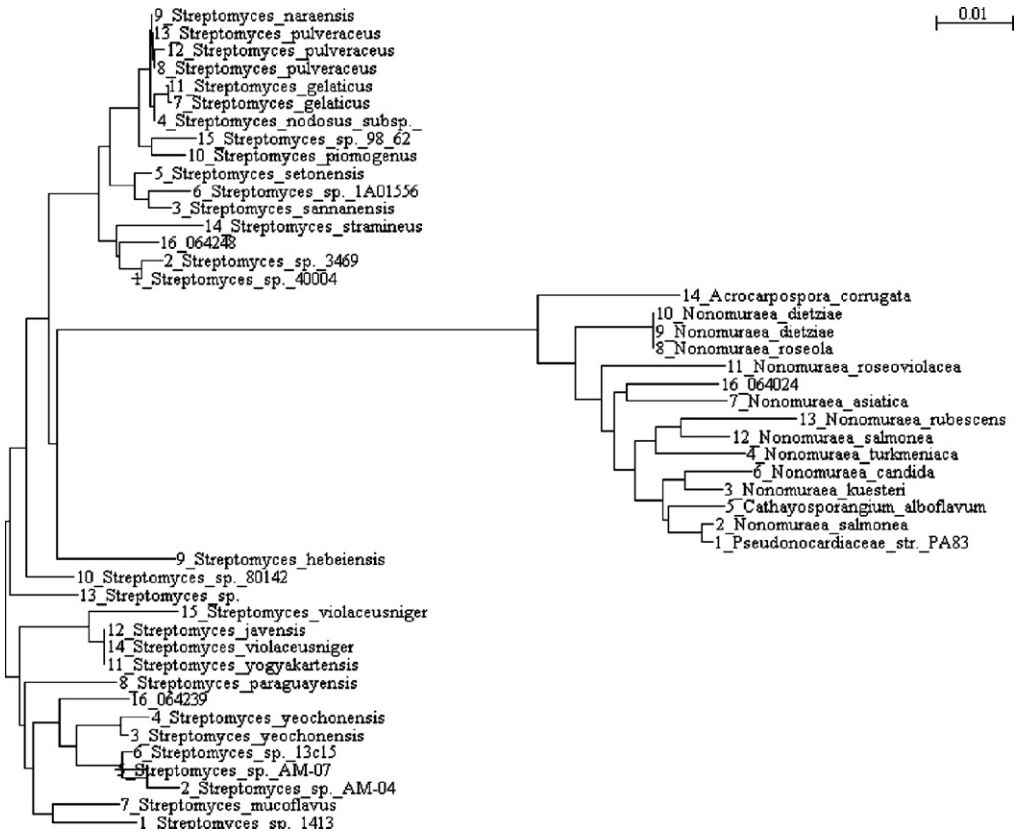

Fig. 5. Evolutionary phylogenetic tree of strains 064024, 064239 and 064248.

064239, 064248 and 065240) was amplified by PCR with primers shown in Table 1, and then the similarity research was performed using NCBI Blastn. The 16S rDNA sequence of strain 064024 agreed 97% with that of *Nonomuraea asiatica*, and strain 064239 agreed 97% with that of *Streptomyces yeochonensis*. The 16S rDNA sequence of strain 064248 agreed 98% with that of *Streptomyces* sp. 40004, and strain 065240 agreed 98% with that of *Rhodococcus* sp. Their evolutionary phylogenetic tree is shown in Figs. 5 and 6.

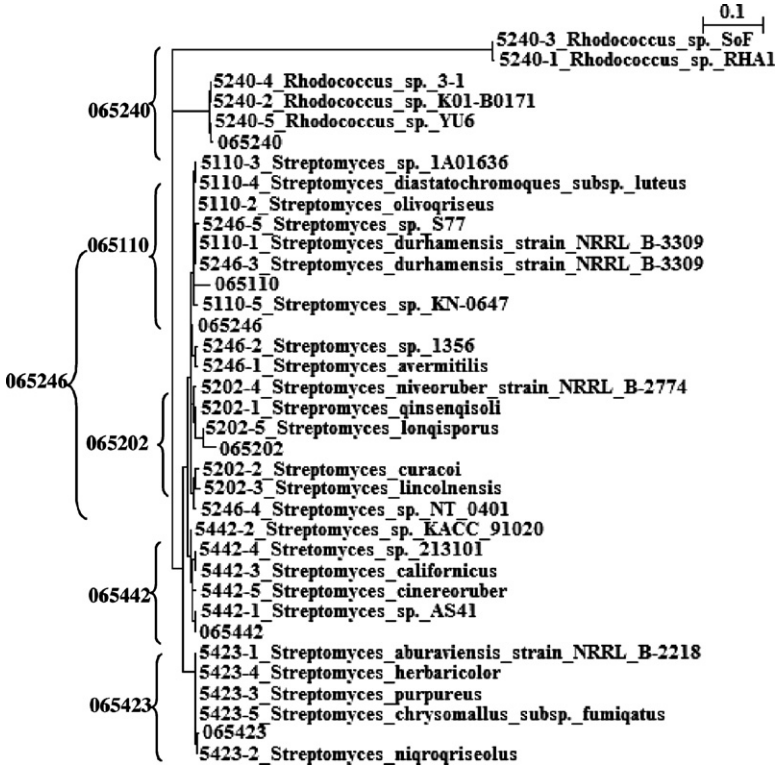

Fig. 6. Evolutionary phylogenetic tree of strain 065240.

3. Conclusion

We have found that ethyl difluoroacetate (DFAc) was accumulated by bacteria, giving difluoroacetic acid and then the carbon–fluorine bonds were cleaved to form fluoride ion. We have succeeded the isolation and purification of bacteria which was possible to accumulate fluorobenzene and/or benzotrifluoride.

4. Experimental

4.1. General

All commercially available reagents were used without further purification. Chemical shifts of ^{1}H (500 MHz) and ^{19}F NMR (470 MHz) spectra were recorded in ppm δ downfield from the following internal standard (Me_4Si, δ 0.00) in $CDCl_3$. The 16S rDNA of strains was amplified by PCR with primers and Ex Taq polymerase (Takara), and similarity research was performed using NCBI Blastn. CEQ8000 DNA analysis (BeckmanCoulter) was used for the sequence.

4.2. Screening of bacteria for degradation

Into a test tube production medium (4 ml) derived from starch (0.5%), sucrose (0.5%), N.Z. Amine (0.25%), peptone (0.25%), yeast extract (0.2%), extract ehlich (0.1%), KH_2PO_4 (0.1%), $MgSO_4{\cdot}7H_2O$ (0.05%) and buffer solution consisted of Na_2HPO_4 and KH_2PO_4, 200 μl of culture was inoculated. Into the above test tube, fluorinated material such as EDFA, FB and/or BTF (4–16 mM) was added, and then the degradation of fluorinated materials was carried out at 28 °C for 2 weeks aerobically.

Cells were collected by centrifugation and removed by filtration. Concentration of fluoride ion in the filtrate was detected by ISE combination fluoride (ION pH/mV/ORP; Mettler-Toledo Group, Swiss).

4.3. Isolation

After being carried out the transformation of ethyl difluoroacetate at 28 °C for 14 days aerobically, the NMR spectra were measured. In the ^{19}F NMR spectrum, the coupling patterns of fluorine (doublet, $J_{F\text{-}Hgem}$ = 53.4 Hz at δ 34.6 ppm and doublet, $J_{F\text{-}Hgem}$ = 53.4 Hz at δ 35.1 ppm from internal C_6F_6 in D_2O) support the interaction of protons with the CF_2HX group. ^{1}H NMR spectrum have four signals containing two CHF_2 proton atoms (triplet, $J_{H\text{-}Fgem}$ = 53.4 Hz at δ ppm; triplet, $J_{H\text{-}Fgem}$ = 53.4 Hz at δ ppm) and CH_3CH_2 proton atom. From the results of ^{1}H and ^{19}F NMR, we have determined that the structures of fluorinated materials are ethyl difluoroacetate and difluoroacetic acid.

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