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Degradation of carbonyl sulfide by Actinomycetes and detection of clade D of β -class carbonic anhydrase

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One sentence summary: Actinomycetes degraded 30 ppmv COS, some bacteria emitted COS in ambient air, and *Mycobacterium* spp. and *Williamsia* sp. had partial nucleotide sequences of clade D gene of β -class carbonic anhydrase.

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ABSTRACT

Carbonyl sulfide (COS) is an atmospheric trace gas and one of the sources of stratospheric aerosol contributing to climate change. Although one of the major sinks of COS is soil, the distribution of COS degradation ability among bacteria remains unclear. Seventeen out of 20 named bacteria belonging to Actinomycetales had COS degradation activity at mole fractions of 30 parts per million by volume (ppmv) COS. *Dietzia maris* NBRC 15801^T and *Mycobacterium* sp. THI405 had the activity comparable to a chemolithoautotroph *Thiobacillus thioparus* THI115 that degrade COS by COS hydrolase for energy production. Among 12 bacteria manifesting rapid degradation at 30 ppmv COS, *D. maris* NBRC 15801^T and *Streptomyces ambofaciens* NBRC 12836^T degraded ambient COS (~500 parts per trillion by volume). *Geodermatophilus obscurus* NBRC 13315^T and *Amycolatopsis orientalis* NBRC 12806^T increased COS concentrations. Moreover, six of eight COS-degrading bacteria isolated from soils had partial nucleotide sequences similar to that of the gene encoding clade D of β -class carbonic anhydrase, which included COS hydrolase. These results indicate the potential importance of Actinomycetes in the role of soils as sinks of atmospheric COS.

Keywords: carbonyl sulfide degradation; Actinomycetes; atmospheric trace gas; β -class carbonic anhydrase; carbonyl sulfide hydrolase

INTRODUCTION

Carbonyl sulfide (COS) is the most abundant sulfur compound in the atmosphere, with about 500 parts per trillion by volume (pptv) present in the troposphere (Torres et al. 1980; Bandy et al. 1992; Chin and Davis 1995; Andreae and Crutzen 1997). Some of the COS in the troposphere is transported to the stratosphere and degraded to sulfate, which is a source for the formation

of stratospheric aerosols, thus influencing Earth's radiation and ozone depletion (Baldwin et al. 1976; Crutzen 1976; Turco et al. 1980; Chin and Davis 1993). Furthermore, COS is regarded as a tracer for quantitating of photosynthetic CO₂ flux (Campbell et al. 2008; Seibt et al. 2010; Stimler et al. 2010). Therefore, studies on the budget of atmospheric COS are important.

Soil is the second largest sink of COS after vegetation, but estimated soil COS uptake values are still uncertain (Watts 2000;

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Launois et al. 2015). In plants, β -class carbonic anhydrase (β -CA), which reversibly hydrates CO_2 to HCO_3^- , contributes to COS degradation because COS is a structural analog of CO_2 (Protoschill-Krebs, Wilhelm and Kesselmeier 1996). Moreover, studies of soil that has been autoclaved and treated with a CA inhibitor showed that the main process of degradation in soil was biological, and that CA was involved in part of this process (Kesselmeier, Teusch and Kuhn 1999; Saito et al. 2002), though recent studies show abiotic COS production in soil by increases of temperature and light (Whelan and Rhew 2015; Whelan et al. 2016). Although some microorganisms are known to degrade COS, the degradation activities of various types of microorganisms are still poorly understood (Smith and Kelly 1988; Smith et al. 1991; Katayama, Kanagawa and Kuraishi 1993; Kato et al. 2008; Li et al. 2010; Kusumi, Li and Katayama 2011). In our previous study, the vertical gradient of COS concentration near the ground surface suggested the role of soil as sinks of atmospheric COS (Kato et al. 2012). Soil bacteria belonging to the genus *Mycobacterium* degraded COS at both ppmv level and atmospheric concentrations, whereas bacteria of the genera *Williamsia* and *Cupriavidus* were unable to degrade the latter (Kato et al. 2008). Thus, it is likely that Actinomycetes such as the bacteria in the genus *Mycobacterium* can degrade atmospheric COS in soil.

Recently, enzymes homologous to β -CA and with high degradation activity have been purified from *Acidianus* sp. strain A1-3, *Acidithiobacillus thiooxidans* strains S1p and G8 (all CS₂ hydrolase) and from *Thiobacillus thioparus* THI115 [COS hydrolase (COSase)] (Smeulders et al. 2011, 2013; Ogawa et al. 2013). Although, presence of COS degradation activity in bacterial β -CA has not yet been elucidated, the homology among amino acid sequences of these proteins predicts that bacterial β -CA also has high degradation activity. Therefore, bacteria with high COS-degrading activity may have these enzymes.

Here, we examined and compared the COS-degrading activities of bacteria covering most suborders in Actinomycetales because they are one of major microorganisms in soil (Stackebrandt, Rainey and Ward-Rainey 1997) as well as of *T. thioparus* THI115. Furthermore, we attempted to detect the genes encoding COSase and COSase-related β -CA in COS-degrading bacteria isolated from soil.

MATERIALS AND METHODS

Bacteria and culture conditions

Bacteria shown in Table 1 were used in this study. We examined named bacteria covering most of the order Actinomycetales (eight suborders excluding Actinomycineae and Glycomycineae) of 10 suborders in addition to bacteria isolated in our laboratory from soil. Cells were grown with reciprocal shaking at 120 rpm at 30°C.

Measurement of degradation of COS at 30 ppmv

For heterotrophs, a single colony of each bacterium was inoculated into a glass test tube (20 cm long, 2 cm i.d.) containing 10 mL of PYG (polypeptone-yeast extract-glucose) liquid medium (Kato et al. 2008). Fully grown cells were harvested by centrifugation at 5000 g for 15 min at 20°C, rinsed twice with 25 mM potassium phosphate buffer (pH 7.2), re-suspended in 10 mL of the buffer and the whole amounts were transferred to a glass test tube (20 cm long, 2 cm i.d.). After sealing the tube with a butyl rubber stopper, COS gas (10 300 ppmv in N_2 as a balance gas, Nissan Tanaka, Saitama, Japan) was injected to a headspace

40 mL via a gas-tight microsyringe to make 30 ppmv COS. After 10 min of incubation at 30°C under static conditions for gas diffusion, the bacteria were incubated with reciprocal shaking at 120 rpm at 30°C, and COS in the headspace gas was measured by FPD-GC (GC-14B, Shimadzu, Kyoto, Japan) and a column of Porapak QS (50–80 mesh, Waters Corporation, Milford, MA), as described previously (Katayama et al. 1992; Kato et al. 2008). Some soil isolates accumulated hydrogen sulfide during COS degradation, which interfered with the GC measurement of COS because of the overlapping of peaks. Therefore, we changed the column to β,β' -oxydipropionitrile (Chromosorb W AW-DMCS, GL Sciences, Tokyo, Japan) and flow rate of carrier gas at 17 mL min⁻¹ and the column temperature at 40°C, for the experiments on the bacteria with the prefix THI in Table 1. At the end of the degradation experiment, the total organic carbon (TOC) content of the cells in the test tube was measured with a wet oxidation-non-dispersive infrared gas analyzer (Seto and Tange 1980). The rate constant of COS degradation was defined as k (per hour) of the exponential function $C_{(t)} = C_0 e^{-kt}$, where $C_{(t)}$ is the concentration of COS at time t (hour) and C_0 is the initial COS concentration. For *T. thioparus* THI115, 100 μL of culture fully grown in mTC10 liquid medium (pH 7.2) that consisted of mineral salts and KSCN (Ogawa et al. 2013) was inoculated to 9.9 mL of mTC10 liquid medium. Due to the weak growth, total 50 mL of fully grown cells were used for the measurement as described above.

Measurement of ambient COS degradation

The experiments were performed by using cells grown in PYG liquid medium and on PYG agar medium. Single colony was inoculated into 10 mL of PYG liquid medium in a glass test tube (16 cm long and 1.5 cm i.d.) and cultured for 3–7 days that depended on the bacterium. Fully grown cells were harvested, rinsed and re-suspended as described above. The cell suspension was filtered with a glass fiber filter (GF-75, Advantec Toyo Kaisha, Tokyo, Japan) and then placed on a glass Petri dish (8.8 cm i.d.) containing small amounts of autoclave-sterilized solutions [$[L^{-1}]$: 5.5 mL of 5% (w/v) sodium silicate and 6.0 mL of 1 M potassium dihydrogen phosphate] to prevent drying of the cells. The Petri dish without its lid was then transferred to an aluminum-covered 5-L gas-sampling bag and sealed as described before (Kato et al. 2008).

For the experiment using cells grown on PYG agar medium, single colony was inoculated on to PYG and 10-fold diluted PYG agar media, and incubated for 3–7 days until it was fully grown. The agar plate was then placed in an aluminum-coated 5-L gas-sampling bag and then sealed.

After cryogenic trapping, the concentration of COS in the bag was measured by FPD-GC as described previously (Kato et al. 2008).

Detection of genes encoding COSase and COSase-related β -CA based on amino acid sequence of COSase

Genomic DNA of isolates (THI401–THI405, THI408, THI410 and THI415) was extracted by an ISOPLANT (Nippon Gene, Tokyo, Japan) from cells grown in PYG liquid medium. Degenerate PCR primers (5'-GTNGCNTGYATGGAYGCNMG-3' and 5'-ARCATNCCRCANCKNGTRG-3') were designed on the basis of COSase amino acid sequences that were highly conserved with those of β -CA to detect genes of COSase and COSase-related β -CA (Fig. 2). In addition to the absence of

Table 1. Comparison of COS degradation activity at 30 ppmv and ambient COS.

Phylum	Order	Species ^a	30 ppmv COS			Ambient COS Rate constant (h ⁻¹) ^b
			Rate constant (h ⁻¹)	TOC (mg C)	Rate constant/TOC (h ⁻¹ mg C ⁻¹)	
Actinobacteria	Actinomycetales	Named bacteria in Actinomycetales				
		<i>Dietzia maris</i> NBRC 15801 ^T	2.34 ± 0.49	0.90 ± 0.77	6.19 ± 5.68	> 0.41
		<i>Streptomyces ambofaciens</i> NBRC 12836 ^T	1.57 ± 0.07	1.26 ± 0.06	1.25 ± 0.01	> 0.33
		<i>Geodermatophilus obscurus</i> NBRC 13315 ^T	0.69 ± 0.20	1.24 ± 0.14	0.55 ± 0.10	< -0.05
		<i>Streptosporangium roseum</i> NBRC 3776 ^T	0.55 ± 0.10	1.46 ± 0.16	0.37 ± 0.03	-0.04 ± 0.04
		<i>Gordonia bronchialis</i> NBRC 16047 ^T	0.11 ± 0.01	0.31 ± 0.03	0.35 ± 0.05	0.08 ± 0.18
		<i>Streptomyces albidoflavus</i> NBRC 12854 ^T	0.47 ± 0.04	1.51 ± 0.01	0.31 ± 0.03	0.01 ± 0.02
		<i>Kitasatospora setae</i> NBRC 14216 ^T	0.29 ± 0.01	1.11 ± 0.05	0.26 ± 0.00	-0.09 ± 0.03
		<i>Rhodococcus rhodochrous</i> JCM 2158	0.31 ± 0.00	1.30 ± 0.08	0.24 ± 0.02	0.06 ± 0.07
		<i>Corynebacterium ammoniagenes</i> JCM 1305 ^T	0.23 ± 0.00	1.14 ± 0.22	0.20 ± 0.04	-0.10 ± 0.05
		<i>Nocardoides albus</i> NBRC 13917 ^T	0.31 ± 0.04	1.71 ± 0.02	0.18 ± 0.03	0.09 ± 0.04
		<i>Actinosynnema mirum</i> NBRC 14064 ^T	0.30 ± 0.03	1.99 ± 0.04	0.15 ± 0.01	-0.01 ± 0.01
		<i>Amycolatopsis orientalis</i> NBRC 12806 ^T	0.23 ± 0.04	2.10 ± 0.06	0.11 ± 0.02	< -0.14
		<i>Streptomyces albus</i> NBRC 13014 ^T	0.05 ± 0.01	1.15 ± 0.19	0.05 ± 0.00	n.d.
		<i>Luteococcus japonicus</i> NBRC 12422 ^T	0.06 ± 0.02	1.76 ± 0.00	0.04 ± 0.01	n.d.
		<i>Brevibacterium linens</i> NBRC 12142 ^T	0.07 ± 0.02	2.86 ± 0.01	0.02 ± 0.01	n.d.
		<i>Micrococcus luteus</i> JCM 1464 ^T	0.04 ± 0.02 ^c	2.76 ± 0.07	0.01 ± 0.01	n.d.
		<i>Sporichthya polymorpha</i> NBRC12702 ^T	0.02 ± 0.00 ^c	1.61 ± 0.05	0.01 ± 0.00	n.d.
		<i>Spirillospora albida</i> NBRC 12248 ^T	0.00 ± 0.00	0.38 ± 0.13	0.01 ± 0.00	n.d.
		<i>Skermania piniformis</i> NBRC15059 ^T	0.00 ± 0.00	0.29 ± 0.05	0.01 ± 0.00	n.d.
		<i>Micromonospora chalcea</i> NBRC 13503 ^T	0.00 ± 0.00	1.79 ± 0.04	0.00 ± 0.00	n.d.
		COS-degrading bacteria isolated from soil ^d				
		<i>Mycobacterium</i> sp. THI401	1.27 ± 0.48	0.84 ± 0.32	1.83 ± 1.09	n.d.
		<i>Mycobacterium</i> sp. THI402	0.93 ± 0.09	1.64 ± 0.04	0.57 ± 0.07	n.d.
		<i>Mycobacterium</i> sp. THI403	1.07 ± 0.04	1.98 ± 0.02	0.54 ± 0.01	n.d.
		<i>Mycobacterium</i> sp. THI404	1.70 ± 0.14	1.84 ± 0.10	0.92 ± 0.03	n.d.
		<i>Mycobacterium</i> sp. THI405	3.34 ± 0.14	0.75 ± 0.22	4.62 ± 1.19	n.d.
		<i>Streptomyces</i> sp. THI408	0.20 ± 0.04	1.51 ± 0.09	0.14 ± 0.03	n.d.
		<i>Williamsia</i> sp. THI410	1.00 ± 0.03	1.81 ± 0.04	0.55 ± 0.01	n.d.
Proteobacteria	Burkholderiales	<i>Cupriavidus</i> sp. THI415	0.04 ± 0.01	0.95 ± 0.15	0.04 ± 0.00	n.d.
		COS-degrading bacteria (Chemolithoautotroph)				
		<i>Thiobacillus thioparus</i> THI115 ^e	7.33 ± 2.10	0.88 ± 0.06	8.26 ± 1.84	n.d.

All experiments at 30 ppmv COS were done in duplicate except that *D. maris* NBRC 15801^T and *Mycobacterium* sp. THI401 were done in quadruplicate and quintuplicate, respectively. The experiments at ambient COS were done in triplicate. Values of rate constants and TOC of each organism were shown after subtracted the values of uninoculated control, respectively. Each value showed mean ± standard deviation. The rate constant of *Corynebacterium ammoniagenes* JCM 1305^T at 30 ppmv COS was obtained from the degradation curve up to 5 h as described in Results in detail.

^aThe superscript 'T' indicates a type strain.

^b*Dietzia maris* NBRC 15801^T, *S. ambofaciens* NBRC 12836^T, *G. obscurus* NBRC 13315^T and *A. orientalis* NBRC 12806^T showed mean values because some data obtained by GC analyses were beyond linear range of calibration curve. n.d. = not determined.

NBRC, NITE Biological Resource Center, Tokyo; JCM, Japan Collection of Microorganisms, RIKEN BioResource Center, Ibaraki.

^cAlthough the rate constants that was subtracted from the values of uninoculated control was low, it was considered as COS degrader because there was clear difference in the degradation curves between bacteria-inoculated and un-inoculated control.

^dIsolated by Kato et al. (2008).

^eIsolated by Katayama et al. (1992).

specificity for these enzymes, the primers don't amplify the region of α 5 helix and the extra loop specific to COSase. Therefore, it is unclear whether the resultant sequences are COSase or β -CA. PCR using Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA) was prepared according to the manufacturer's instructions except that each primer was 2 μ M and the template DNA was 10 ng in a total volume of 20 μ L. Thermal cycling was performed at 98°C for 30 s; 25 cycles at 98°C for 10 s, 60°C for 30 s and 72°C for 30 s;

and finally 72°C for 10 min. The PCR products were separated by 2% agarose gel electrophoresis and the expected bands of 185 bp based on COSase gene were extracted by using an illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK). The extract was ligated by a pGEM-T Easy Vector System I (Promega, Madison, WI) and transformed into XL10-Gold competent cells (Agilent Technologies, Santa Clara, CA). PCR product of positive clone was sequenced by a capillary sequencer (3500 Genetic Analyzer, Thermo Fisher Scientific). The nucleotide sequences were

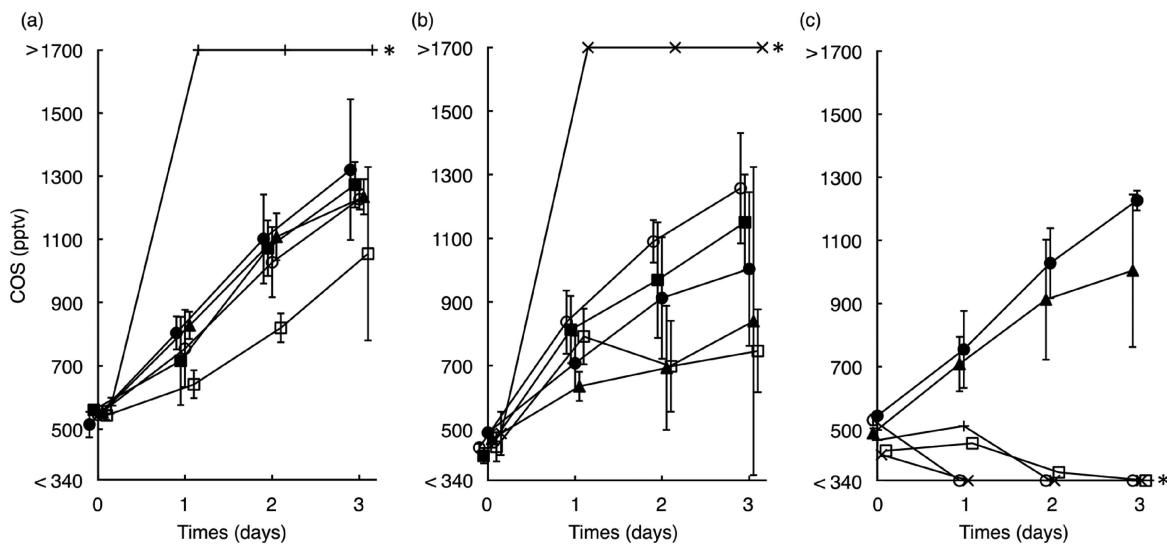


Figure 1. Time courses of ambient COS degradation by Actinomycetes. (a) filled circle, *Streptosporangium roseum* NBRC 3776^T; filled square, *Actinosynnema mirum* NBRC 14064^T; filled triangle, *Streptomyces albidoflavus* NBRC 12854^T; empty square, *R. rhodochrous* JCM 2158; +, *Geod. obscurus* NBRC 13315^T; empty circle, uninoculated control. (b) Empty circle, *Corynebacterium ammoniagenes* JCM 1305^T; filled square, *K. setae* NBRC 14216^T; filled triangle, *G. bronchialis* NBRC 16047^T; empty square, *N. albus* NBRC 13917^T; ×, *A. orientalis* NBRC 12806^T; filled circle, uninoculated control. (c) Empty circle, *D. maris* NBRC 15801^T; ×, + and empty square, *S. ambofaciens* NBRC 12836^T; filled circle and filled triangle, uninoculated control for *D. maris* NBRC 15801^T and *S. ambofaciens* NBRC 12836^T, respectively. The experiments of figures (a) and (b) were separately performed because of the limitation of the manipulation. Figure (c) is shown to emphasize bacteria-degrading ambient COS rapidly. Asterisks indicate the bacteria significantly emitting or degrading COS. All experiments were done in triplicate. The symbol and error bars show mean and standard deviation, respectively, and are offset for clarity. Because results among triplicate time courses of *S. ambofaciens* NBRC 12836^T differed, each assay is separately presented. *A. orientalis* NBRC 12806^T increased COS to 2861 ± 954 pptv in 3 days.

deposited in the DNA Data Bank of Japan under the accession numbers LC127314–LC127318 (for THI401–THI405) and LC127319 (for THI410).

RESULTS

Degradation of 30 ppmv COS

We examined the COS-degrading activities of bacteria covering most suborders in Actinomycetales. Except for *Spirillospora albida* NBRC 12248^T, *Skermania piniformis* NBRC 15059^T and *Micromonospora chalcea* NBRC 13503^T, 17 actinomycetes degraded 30 ppmv of COS (Table 1). Concentration of COS in uninoculated control was also decreased with a rate constant of 0.027 ± 0.003 h⁻¹ ($n = 6$), due to the hydrolysis of COS through monothiocarbonate intermediates to hydrogen sulfide in the presence of H₂O (Ferm 1957; Elliott, Lu and Rowland 1989). Therefore, we defined COS-degrading bacteria as those with rate constants of 0.04 h⁻¹ or more under the condition employed. The degradation curve of *Corynebacterium ammoniagenes* JCM 1305^T did not fit an exponential function at the sampling point of 9 h, and the COS concentration at 17 h that was the final sampling point was 5 ppmv; therefore, the rate constant for this bacterium was estimated based on the degradation curve from 0 to 5 h. We obtained the rate constant per mg of TOC to compare degradation activities (Table 1). Among the tested bacteria, *Dietzia maris* NBRC 15801^T (6.19 ± 5.68 h⁻¹) had the highest degradation activity. Out of the eight isolates from soil, all Actinomycetes had higher activity than that of *Cupriavidus* sp. THI415 (Betaproteobacteria). Low rate constant of *Streptomyces* sp. THI408 may be due to the formation of floc during the cultivation. The value of *T. thioparus* THI115 was high because of the need of COS degradation for the chemolithotrophic energy production.

Degradation of ambient COS

Initial experiment using *Streptomyces ambofaciens* NBRC 12836^T grown in PYG liquid medium revealed an increase in COS from about 500 pptv to about 1300 pptv in 2 days (data not shown). Control PYG liquid medium uninoculated with bacterium also showed emission of COS (to about 1400 pptv) probably because of organosulfur compounds included in yeast extract such as glutathione and cysteine, that is known as COS precursor (Flöck, Andreae and Dräger 1997). These results revealed the difficulty to evaluate accurately the bacterial degradation of COS at low concentrations. To minimize COS emission from the medium, bacteria grown in PYG liquid medium were collected on a filter and assayed after eliminating residual organic substances. As a result, only *D. maris* NBRC 15801^T degraded ambient COS.

We therefore considered that COS degradation by bacteria might require organic compounds such as nutrient medium. Consequently, we chose 12 organisms that showed high rate constants per mg of TOC at 30 ppmv COS, and grew them in 10-fold-diluted PYG medium to lower the interference of abiotic COS emission from the medium. We then performed the ambient COS degradation experiment again (Fig. 1). *D. maris* NBRC 15801^T and *S. ambofaciens* NBRC 12836^T decreased COS. Experiment with *Rhodococcus rhodochrous* JCM 2158, *Gordonia bronchialis* NBRC 16047^T and *Nocardiooides albus* NBRC 13917^T showed the decrease of COS levels compared with those in the uninoculated bag, but the levels were still more than the initial concentrations and the statistical significances weren't indicated in the rate constants between the inoculated and the uninoculated bags ($P > 0.05$ by a two tailed t-test). In contrast, *Geodermatophilus obscurus* NBRC 13315^T and *Amycolatopsis orientalis* NBRC 12806^T clearly increased the concentration of COS. The other bacteria were almost equal with the uninoculated bag. These results suggest

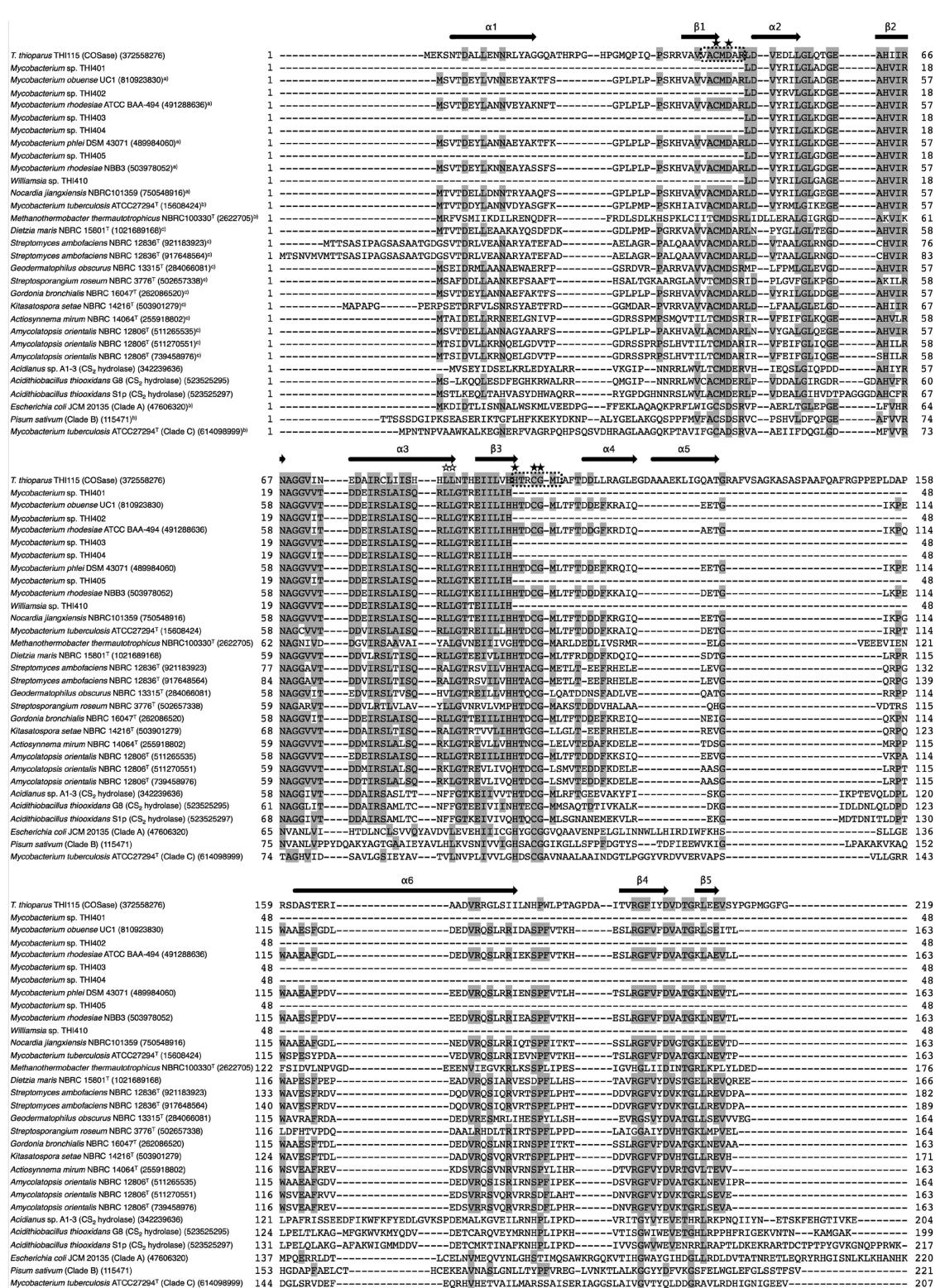


Figure 2. Multiple alignment of amino acid sequences deduced from partial nucleotide sequences of *Mycobacterium* spp. THI401–THI405, and *Williamsia* sp. THI410 with β-CA, COSase and CS₂ hydrolase. The alignment was constructed using ClustalW with MEGA6 (Tamura et al. 2013) and showed using GENETYX-MAC ver. 17 (GENETYX, Tokyo, Japan). The residues to be identical in more than 50% are shown in grey background. All proteins, except as otherwise noted, are clade D of β-CA. The secondary structure of COSase is shown above the alignment. Closed stars show the zinc binding residues and the residues stabilizing the water molecule that occupies the fourth coordination site of COSase (Ogawa et al. 2013). Open stars show the FF motif that is specific to CS₂ hydrolase. The amino acid sequences of COSase used for the design of the degenerate PCR primers surrounded by a broken line. The accession number of each sequence is shown by NCBI GI number in parentheses. (a) Amino acid sequences of β-CA just below each THI401–THI405 and THI410 are those suggesting the highest relatedness by results of NCBI BLASTP search: for example, *Mycobacterium obuense* UC1 for THI401. (b) β-CAs reported their characteristics such as CA activity and X-ray crystal structure in detail. (c) Included in this figure, because Clade D of β-CA was found in the genome databases of Actinomycetes used here.

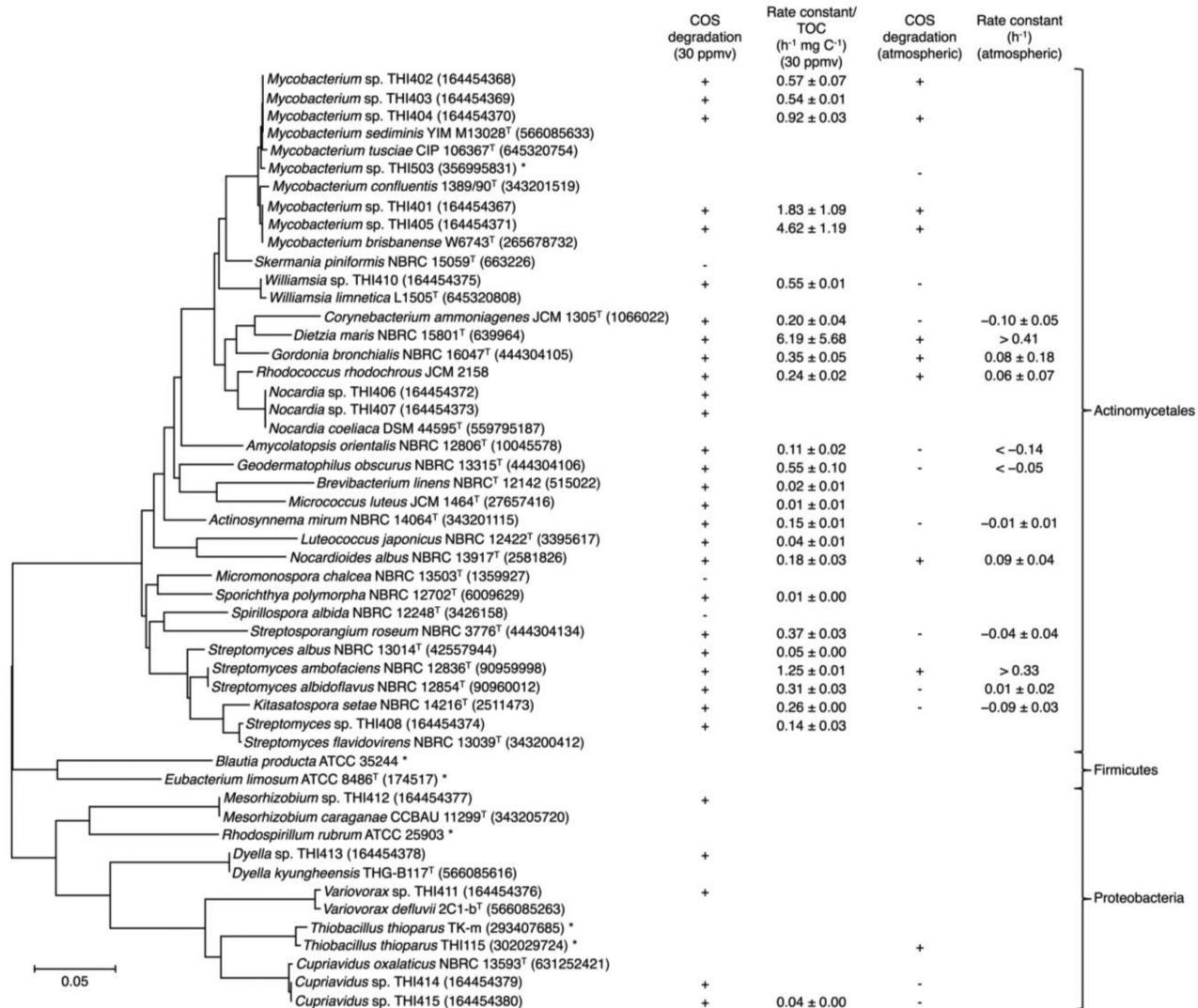


Figure 3. The relationship of COS degradation activity and their phylogenetic position based on 16S rRNA gene of bacteria used in this study. The phylogenetic tree was constructed using the neighbor-joining method with MEGA6 (Tamura et al. 2013). *Nocardia* sp. THI406, *Nocardia* sp. THI407, *Variovorax* sp. THI411, *Mesorhizobium* sp. THI412 and *Dyella* sp. THI413 are COS-degrading bacteria isolated in the research of Kato et al. (2008) and their phylogeny were determined. Bacteria that phylogenetically related to isolates by Kato et al. (2008) are also shown in the phylogenetic tree. Bacteria marked with an asterisk are known as COS-degrading bacteria (Smith and Kelly 1988; Smith et al. 1991; Katayama, Kanagawa and Kuraishi 1993; Kusumi, Li and Katayama 2011; Smeulders et al. 2013). The accession numbers of 16S rRNA gene sequence data are shown by NCBI GI number in parentheses. Due to the lack of 16S rRNA gene sequence data, 16S rRNA genes of *Blautia producta* JCM 1471^T (GI: 631252072), *Rhodospirillum rubrum* ATCC 11170^T (GI: 444303827) and *R. rhodochrous* DSM 43241^T (GI: 640003) were used from the data of *Blautia producta* ATCC35244, *Rhodospirillum rubrum* ATCC 25903 and *R. rhodochrous* JCM 2158, respectively. *Blautia producta* ATCC35244 was formerly classified as *Peptostreptococcus productus* strain U-1 in the research of Smith et al. (1991).

that only limited number of bacteria can degrade ambient COS under the experimental conditions employed.

Detection of gene encoding β -CA

Nucleotide sequences of 145 bp similar to those of the β -CA gene were detected from at least four positive clones from *Mycobacterium* spp. THI401, THI402, THI403, THI404, THI405 and *Williamsia* sp. THI410 (Fig. 2). However, the sequences of some positive clones deduced different proteins, suggesting low specificity of primers. *Streptomyces* sp. THI408 did not produce an amplified band of the expected size (185 bp) on agarose gel electrophoresis. *Cupriavidus* sp. THI415 produced the band with the expected size, however, the sequences of the candidate 12 positive clones

involved completely different proteins such as cellulose synthase, lauroyl acyltransferase and DNA gyrase (data not shown). However, the possibility of the presence of β -CA or COSase in *Streptomyces* sp. THI408 and *Cupriavidus* sp. THI415 can't be ruled out because of the specificity of primer designed or PCR condition employed.

DISCUSSION

In contrast to the experiment at 30 ppmv COS, only two bacteria had clear ambient COS degradation activity. The activity of ambient COS degradation of *S. ambofaciens* NBRC 12836^T changed with the experimental conditions such as growth in nutrient medium, indicating that the COS degradation

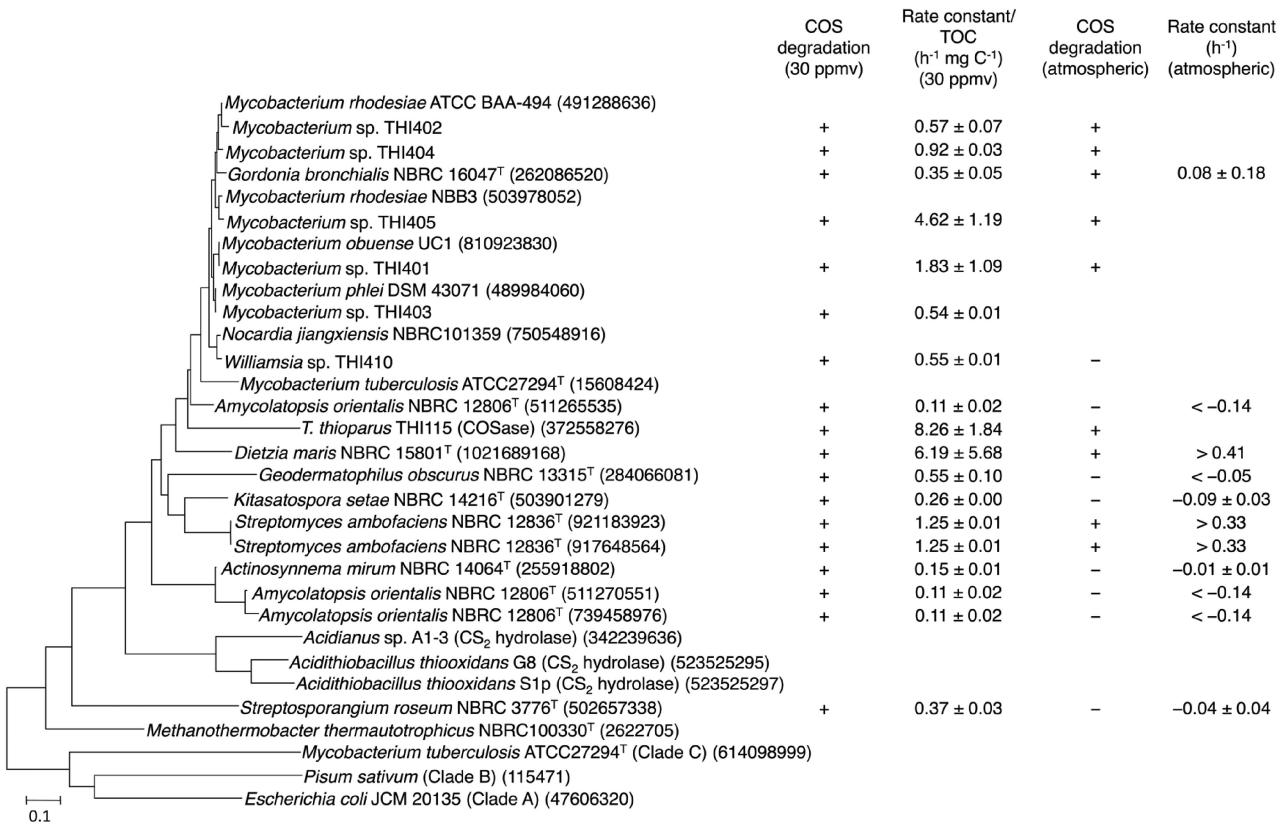


Figure 4. Phylogenetic tree based on amino acid sequences of β -CA, COSase and CS₂ hydrolase and their relation to the COS-degrading activity. Enzyme proteins that were illustrated in Fig. 2 were used to construct the phylogenetic tree using the neighbor-joining method with MEGA6 (Tamura et al. 2013).

activity of this bacterium depends on various factors which affect on the physiological conditions of bacteria. Like the compensation points of COS observed in some soils (Lehmann and Conrad 1996; Kesselmeier, Teusch and Kuhn 1999; Conrad and Meuser 2000), the bacteria that degraded 30 ppmv COS but not ambient COS may also have compensation points or thresholds of COS degradation within the range of these concentrations. COS production in some soil, and the rhizosphere of some plants are also reported (Melillo and Steudler 1989; Kanda, Tsuruta and Minami 1992, 1995; Liu et al. 2010; Whelan, Min and Rhew 2013; Maseyk et al. 2014; Whelan and Rhew 2015; Whelan et al. 2016). In soils or microsites that involves higher concentration of COS than the atmosphere, bacteria that do not degrade ambient COS may also contribute to COS degradation. On the other hand, *Geod. obscurus* NBRC 13315^T and *A. orientalis* NBRC 12806^T produced COS under ambient COS. COS production by microorganisms has been known in degradations of thiocyanate and CS₂ (Smith and Kelly 1988; Katayama et al. 1992; Sorokin et al. 2007; Smeulders et al. 2011, 2013; Hussain et al. 2013), however, it was not in the case because these compounds were not added in the system. Therefore, the increase may be attributed to organosulfur compounds known as COS precursor (Flöck, Andreae and Dräger 1997).

It is natural for *T. thioparus* THI115 to have high COS-degrading activity because of its energy requirement. On the other hand, the reason of high activities found in *D. maris* NBRC 15801^T and *Mycobacterium* sp. THI405 is not well understood because these species are known generally as chemoorganotrophs. CD-Search, tool searching the NCBI's conserved domain database, indicated that β -CAs showing the highest relation-

ship to nucleotide sequences revealed in this study belong to clade D that is one of four phylogenetically distinct clades of β -CA. Clade D genes were also found in the genome database of some Actinomycetes used in this study (Fig. 2). Although COSase and CS₂ hydrolase are family enzymes of clade D, these clade D lack the amino acid sequences corresponding to α 5 helix and the extra loop (Gly150–Pro158) of COSase and FF motif of CS₂ hydrolase that could be responsible for the degradation activity against COS and CS₂, respectively (Smeulders et al. 2011, 2013; Ogawa et al. 2013) (Fig. 2). Thus, COS-degrading reaction by these chemoorganotrophs may only accompany reaction between CO₂ and HCO₃[–] by β -CA as well as the case found in plants (Protoschill-Krebs, Wilhelm and Kesselmeier 1996). However, *Mycobacterium* spp. have been known for carrying facultative chemolithoautotrophic sulfur-oxidizing ability, and *Mycobacterium* sp. THI503 had COS degradation activity (Kusumi, Li and Katayama 2011). Therefore, COS degradation activity in *Mycobacterium* spp. may be linked to energy production. We created phylogenetic trees on the basis of 16S rRNA gene and β -CA (Figs 3 and 4). There is no clear relationship between the rate constants per mg of TOC and the phylogenetic position.

We showed here that many Actinomycetes had COS-degrading activity at 30 ppmv COS and that some of them also had activity at ambient levels of COS. In addition, presence of β -CA in the Actinomycetes suggests the contribution of these microorganisms and enzymes involved in one of the roles of COS sink. Future experiment on the distribution of β -CA carrying COS-degrading activity in soil microorganisms including fungi (Masaki et al. 2016) is needed for better understanding soil microorganisms as an important sink of COS. Furthermore,

experiment on microbial COS production is needed for the accurate estimation of COS flux between terrestrial environment and atmosphere.

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Conflict of interest. None declared.

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