水田土壌から単離した微生物によるイプコナゾールの分解

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Original Article

Degradation of Ipconazole by Microorganisms Isolated from Paddy Soil

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Ipconazole is a triazole fungicide for treating rice seed. In the present study, degradation of ipconazole by isolated microorganisms from paddy soil was investigated. To enrich the degraders, the soil sample was perfused with ipconazole solution for 41 days. After the perfusion, the ipconazole-tolerant microbes in the perfused soil were cultivated on various media containing ipconazole. One bacterial, 12 actinomycetous, and 7 fungal strains showed ipconazole-degrading activity among 39 strains of bacteria, 14 strains of actinomycetes, and 14 strains of fungi in the liquid media containing 0.1 μg/ml ^{14}C-ipconazole after 28 days of incubation. In particular, 8 strains of actinomycetes decomposed more than 90% of the ipconazole. The metabolism of ipconazole was investigated using two strains of actinomycetes, A1 and D16, with a higher level of degrading activity than the others. The isolates A1 and D16 were identified as *Kitasatospora* sp. and *Streptomyces* sp., respectively. A1 degraded more than 80% of the applied ipconazole after 3 days incubation in a liquid culture containing ^{14}C-ipconazole at 1 μg/ml. D16 degraded approximately 20% of the applied ipconazole after 2 days, but more than 99% after 6 days of incubation. The primary metabolic reaction could be dominated by oxidation at either the carbon of the methine in the isopropyl group or the carbon of the benzylmethylene. A1 and D16 also possess the ability to oxidize the carbon of the methyl portion of the isopropyl group as well as that of the methylene portion of the cyclopentane ring. The polar metabolites in ethyl acetate extracts had increased by the end of incubation. 1,2,4-Triazole was detected as a water-soluble metabolite in the culture. There is little information available regarding microbial degradation of azole compounds, but our results suggest that some soil microorganisms contribute to the biodegradation of the triazole fungicide ipconazole in soil.

Key words: degradation, metabolite, ipconazole, triazole fungicide, soil microorganism.

INTRODUCTION

Ipconazole, (1*RS*,2*SR*,5*RS*; 1*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol, is a triazole fungicide for the treatment of rice seed that was discovered by Kureha Chemical Industry Co., Ltd. The fate of ipconazole in rice plants has been reported in our previous paper.[1] We have found that 3 weeks after the treatment of rice seed with ipconazole, approximately 30% of the ipconazole or its metabolites can be recovered from the rice plants, whereas 40% of the ipconazole is found in the soil in the nursery boxes. The depletion process for ipconazole in soil, however, has not yet been studied. The depletion of pesticides in soil may be governed by the sorption of chemicals by soil particles and by the availability of pesticides to the degrading microorganisms. S. G. Patil *et al.*[2] have reported the effects of structure and physicochemical properties on the rate of degradation of triazole compounds in soil. They have shown that the electron-withdrawing substituents and lipophilic groups in 1-benzyltriazoles increase adsorption by soil and enhance the persistency of triazole compounds in soil. Recently, Céline *et al.*[3] have reported that the moisture content of soil affects the sorption by loam soil of hydrophobic inhibitors (flusilazole, propiconazole, and epoxiconazole) of the biosynthesis of sterol. They adopted Wershaws humus model and assumed that a low moisture content would modify the structure of humic substances and generate hydrophilic surfaces, which favor the sorption of the above hydrophobic fungicides. Under the guidelines of the Ministry of Agriculture, Forestry, and Fisheries of Japan, it was found that the DT_{50} values of ipconazole in a beaker test using paddy soils under flooded conditions are 76–80 days, and in upland soils, 45–54 days (unpublished data). The hydrophobicity of ipconazole may participate in the depletion process. In

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contrast, there have been few reports on the biological degradation of triazole fungicide in soil. N. Mikami *et al.* have reported the metabolism of diniconazole by a microorganism isolated from soil.[4] The reduction of triadimefon to triadimenol carried out by many species of fungi has been well recognized.[5-7] In the present study, to further understand the bioavailability of ipconazole in soil, the soil microorganisms that degrade ipconazole were screened from paddy soil. The taxonomical characteristics of some isolates and their metabolic pathways for ipconazole are discussed herein.

MATERIALS AND METHODS

1. Chemicals

Ipconazole is a mixture of geometric isomers, isomer *cc* ((1*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,-4-triazol-1-ylmethyl)cyclopentanol) and isomer *ct*((1*RS*,-2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl) cyclopentanol).[1] [Triazole-^{14}C]-ipconazole (2140 MBq/mmol, radiochemical purity >99%, isomer *cc*/ isomer *ct* ratio 98.6/1.4) was synthesized by Amersham International Plc., U.K. and purified on a reverse-phase silica gel Lobar column (Lichroprep RP-18, 10 mm×240 mm, Merck) using methanol/water (5/1) as an eluant. Unlabeled ipconazole (isomer *cc* 88.7%, isomer *ct* 10.6%), isomer *cc* (chemical purity 99.6%), isomer *ct*, and authentic metabolites (M1, M4, M5, M6, M11–M13, and M23) were prepared in our laboratory.[1] Isomer *cc* was used for the degradation studies as the predominant geometric isomer of ipconazole. The standard M22 (1,2,4-triazole) was purchased from Tokyo Kasei Co., Ltd.

2. Soil Sample

Fresh soil samples were collected from a paddy field in Nakoso, Iwaki, where ipconazole had not been applied. The sample was dried at room temperature and passed through a 5-mm sieve. The soil properties were as follows: texture, light clay; pH(H_2O), 6.0; total carbon, 2.67%; and cation exchange capacity, 19.6 meq/100 g.

3. Soil Perfusion

Twenty grams of the sample soil was placed in a glass column[8] (3 cm i.d. × 20 cm) and perfused with distilled water for 6 days, with 0.1 μg/ml isomer *cc* solution for 22 days, and with 0.2 μg/ml isomer *cc* solution for 19 days, successively. These concentrations were chosen to minimize the influence on the growth of microflora.

4. Isolation and Identification of Ipconazole-Degrading Soil Microorganisms

One gram of soil was collected from the top layer of the column before and after perfusion. The soil suspension was prepared by sequential dilution with a sterilized 0.85% NaCl solution, and the samples of appropriate soil dilutions were spread on plates. The aerobic bacterial and actinomycetous colonies were enumerated on an egg-albumin agar medium[9] after 8 days of incubation at 30°C. The fungal colonies were enumerated on rosebengal agar medium[9] after 5 days of incubation at 25°C.

The colonies of bacteria, actinomycetes, and fungi grown on each medium were randomly transferred to the corresponding medium containing 1, 10, or 100 μg/ml isomer *cc*. Ipconazole-tolerant microorganisms grown on these media were isolated by the successive transfer of a single colony.

Bacterial strains were inoculated into 4 ml of the nutrient broth containing [triazole-^{14}C]-ipconazole at 0.1 μg equiv./ml and incubated with shaking at 28°C for 28 days. Actinomycetous and fungal strains were inoculated into 4 ml of egg-albumin and Czapek-dox media,[9] respectively, with [triazole-^{14}C]-ipconazole at 0.1 μg equiv./ml. The none biotic depletion was checked in each medium without inoculation. After 28 days of incubation at 28°C, these liquid media were acidified to pH 2 and shaken with 6 ml of methanol. These samples were filtered through a glass fiber filter (GF/C, Whatman). The filtrate was concentrated *in vacuo* or under nitrogen gas and extracted twice with 4 ml of ethyl acetate. The extracts were dried with anhyd. Na_2SO_4, and the concentrates were subjected to TLC. When the degradation of ipconazole was at least 20% greater than that in the media without inoculation, the strain was confirmed as an ipconazole degrader.

The taxonomy of some actinomycetous and fungal strains with ipconazole-degrading activity has been studied by morphological observation and biochemical methods.[10-14] Regarding the isolates A1 and D16 in actinomycetes, analyses of menaquinones,[12] the cell-wall chemotype,[13] and the utilization of carbon source[14] were carried out by Japan Food Research Laboratories (Tokyo).

5. Isolation and Identification of Ipconazole Metabolites

The D16 isolate was inoculated into 50 ml of yeast extract/malt extract medium (yeast extract 4 g/l, malt extract 10 g/l, glucose 4 g/l, pH 7.0), designated as YM medium, in a 300-ml glass flask and incubated on a rotary shaker for 3 days at 28°C. Then, 20 ml of this cultural solution was inoculated with 2l of YM medium in 3-l jar fermenters (Biomaster D, 28°C, 400 rpm, 1 vvm). After a 2-day incubation, unlabeled isomer *cc* dissolved in dimethyl sulfoxide (DMSO) was added at a final concentration of 20 mg/l and then incubated for 7 more days. The 4l of cultural solution was passed through filter paper. The mycelia were extracted with methanol over night, and the extract was separated by centrifugation (4700 *g*, 10 min). After the supernatant was concentrated *in vacuo*, it was combined with the culture filtrate. The combined filtrate was extracted twice with ethyl acetate. This ethyl acetate fraction was concentrated *in vacuo* and chromatographed on a silica gel (Wako gel C-300, Wako pure chemical industry Co., Ltd.) and eluted with 400 ml each of various chloroform/methanol solutions (100/0, 99/1, 95/5, 90/10 and 0/100). The fraction of

the 95/5 solution was then applied to a Lobar column (Lichloprep RP-18, type A, 240 mm × 10 mm, E. Merck, Germany) and eluted with 150 ml each of various methanol/water solutions (30/70, 50/50, 70/30 and 100/0). The concentrated fraction of the 70/30 solution was chromatographed on a silica gel and eluted with a benzene/acetone solution (2/1). A 15 ml aliquot of eluent was collected, and three metabolites, AM2, AM3, and AM4, were purified by preparative ODS-column chromatography (Inertsil ODS-2, 10.7 mm × 250 mm, GL Science) using a Shimadzu LC-6AD solvent delivery system equipped with a SPD-6A (Shimadzu) for the UV detector, with acetonitrile/water (45/55) used as the eluent at a rate of 4 ml/min. These metabolites were subjected to MS and NMR.

6. Metabolic Study Using ^{14}C-Ipconazole and Isolated Soil Microorganisms

The metabolism of ipconazole was studied using the isolates A1 and D16, which showed stronger degrading activity than the others. Each isolate was incubated with shaking for 3 days in YM medium at 28°C. Ten milliliters of the culture solution was transferred to Petri dish A (diameter: 6 cm; height: 2 cm) and supplemented with 10 μg equiv. ^{14}C-ipconazole. This dish was put into Petri dish B (diameter: 9 cm; height: 6 cm) containing 15 ml of 2.5 N NaOH solution. A polyurethane foam was attached to the underside of the lid of Petri dish B and used to absorb volatized metabolites. The volatized metabolites were extracted from the polyurethane foam with toluene. The culture solutions were periodically collected and extracted as described in *4*. These extracts and the water-soluble fractions were subjected to HPLC and TLC analysis, respectively.

7. Radioassay

The radioactivity in solvent extracts was measured with a liquid scintillation counter (LSC-700, Aloka).[1)]

8. TLC

Liquid fractions were analyzed with precoated high-performance TLC plates (HPTLC silica gel HF_{254}, 10 × 10 cm, 0.2-mm thickness, E. Merck) using benzene/acetone (2/1) as an eluant.[1)] The radioactivity was detected and quantified with a Radiochromanizer (Aloka). The locations of M22 were detected by heating at 110°C for 10 min after spraying 0.1% $CuSO_4$ (w/v) in water.

9. HPLC

HPLC was performed using a Shimadzu LC-9A solvent delivery system (Shimadzu), a UV detector (TOYOSODA UV800, TOYO SODA Co., Ltd.), and a radioactivity detector (RLC-551, Aloka). An ACM-550 (Aloka) was used as the operating system. The column (Inertsil ODS-2, 4.6 mm × 250 mm, GL Science) was used with an acetonitrile/water (45/55) solvent system up to 34 min, and acetonitrile/water (75/25) from 34 min to 60 min, at a rate of 0.8 ml/min.

RESULTS

1. Isolation of Ipconazole-Degrading Microorganisms in Soil after Soil Perfusion

The populations of bacteria, actinomycetes, and fungi in perfused soil are shown in Table 1. Among the isolated microorganisms, 39 bacterial strains, 14 strains of actinomycetes, and 14 strains of fungi were randomly chosen to test the ipconazole-degrading activity. Ipconazole-degrading activity was found in 1, 12, and 7 strains of bacteria, actinomycetes, and fungi, respectively.

2. Degrading Activity and Taxonomic Properties of Ipconazole-Degrading Microorganisms

The activities of the screened degraders are shown in Table 2. Among the strains of actinomycetes, eight decomposed more than 90% of the ipconazole in 28 days. The strains of fungi degraded 40–85% of the ipconazole. The bacterial strain exhibited a lower level of degrading activity than did the degraders of actinomycetes and fungi.

The two strains (A1 and D16) of actinomycetes with a higher level of degrading activity than the others were used for further taxonomic and metabolic studies. Morphological

Table 1. The change in the total population of microorganisms in soil before and after perfusion

Microorganisms	Population (cfu/g dry soil)		
	Before perfusion	After perfusion	
	untreated	untreated control [a)]	treated with ipconazole [b)]
Bacteria	2.3×10^7	1.0×10^7	3.5×10^7
Actinomycetes	1.7×10^6	1.2×10^6	1.8×10^6
Fungi	5.9×10^5	1.9×10^5	2.7×10^5

[a)] The soil was perfused with distilled water.
[b)] The soil was perfused with ipconazole solution.

Table 2. Degrading activity of the ipconazole-degrading microorganisms isolated from soil

Microorganisms	Number of microorganisms degrading ipconazole			
	20%-30% [a]	30%-60%	60%-90%	more than 90%
Bacteria (39) [b]	1	0	0	0
Actinomycetes (14)	0	3	1	8
Fungi (14)	0	4	3	0

[a] Degradation of the ^{14}C-ipconazole applied at 0.1 μg/ml after 28 days incubation. [b] Figures in parentheses show the number of strains tested for ipconazole-degrading activity.

observations were made using a light microscope and a scanning electron microscope. The electron micrographs of the tested microorganisms are shown in Fig. 1. The characteristics of A1 and D16 are summarized in Table 3. Regarding the isolate A1, the aerial mycelium formed a straight chain of many smooth-surfaced spores, and the cell wall hydrolysate contained LL-diaminopimeric acid (spores) and meso-diaminopimeric acid (mycelia). The major type of menaquinone in A1 was MK-9 (H_6, H_8). These results suggest that A1 belongs to the genus *Kitasatospora*.[15] The isolate D16 showed the same characteristics as A1, but LL-diaminopimeric acid was contained in the cell wall hydrolysate. Therefore, D16 was classified into the genus *Streptomyces*.[16]

Among the ipconazole-degrading fungi, F1 and F7 were identified as *Mucor* sp. and *Penicillium* sp., respectively, based on microscopic observation.[13,14] The other fungi have not been identified.

3. Isolation and Identification of Ipconazole Metabolites

In the preliminary study of ipconazole metabolites, unidentified metabolites were found in the culture of isolate A1 as well as that of D16. However, the quantity of each metabolite from the culture of A1 was insufficient for isolation due to its rapid degradation. To identify the unknown metabolites of ipconazole converted by the actinomycetes, the isolate D16 was cultured with 20 mg/l of unlabeled isomer *cc* in a jar fermenter. Most of the metabolites in the D16 culture were found in a chloroform/methanol (95/5) fraction using silica gel chromatography. Five metabolites of ipconazole which showed the same retention times on HPLC with authentic compounds (M1, M5, M6, M12 and M13) were found in the D16 culture. Among the unknown metabolites, three were isolated and designated as AM2, AM3, and AM4.

The metabolite AM2 was identified as (1*RS*,2*SR*,5*RS*)-2-[(4-chlorophenyl)-hydroxymethyl]-5-(1-hydroxy-1-methyl-

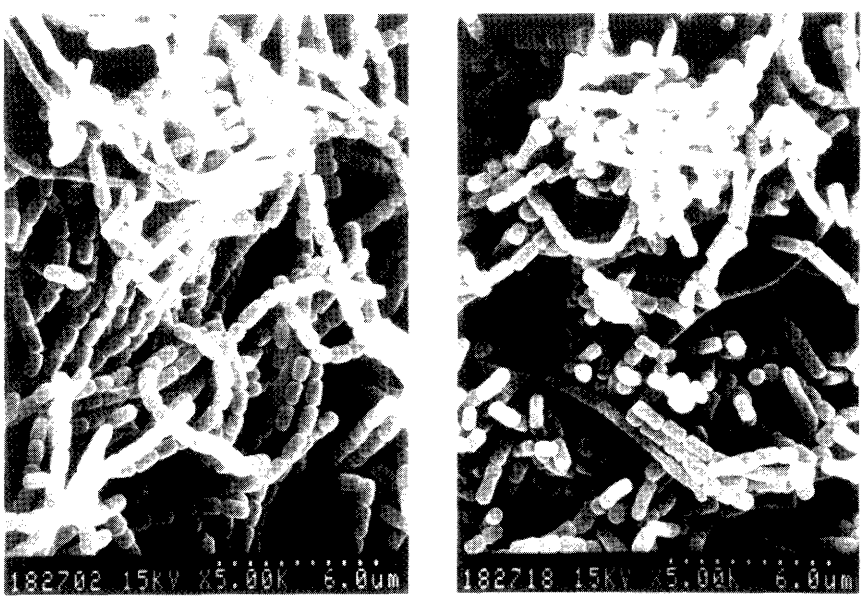

Fig. 1. Scanning electron micrographs of spores of ipconazole-degrading actinomycetes A1 and D16.

Table 3. Characteristics of ipconazole-degrading actinomycetes A1 and D16

Characteristics	A1	D16
Morphology of sporulation	long chains	long chains
Cell wall chemotype	III[a] [I][b]	I
LL-Diaminopimeric acid	- [+]	+
meso-Diaminopimelic acid	+ [-]	-
Diaminobutyric acid	-	-
Glycine	trace	+
Aspartic acid	-	-
Ornithine	-	-
Lysine	-	-
Arabinose	-	-
Galactose	+	+
Menaquinones	MK-9(H_6, H_8)	MK-9(H_6, H_8)
Utilization of carbon		
Arabinose	+	+
Xylose	+	+
Inositol	-	-
Mannitol	-	-
Fructose	-	weak
Rhamnose	-	-
Sucrose	-	-
Raffinose	-	-
Melezitose	-	-

[a] Mycelia. [b]Spores.

ethyl)-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol (Fig. 2), based on the following evidence: ^{1}H NMR (500 MHz, $CDCl_3$) δppm, 1.22–1.26 (m, 1H, H-5 of cyclopentanol), 1.32 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.57–1.61 (m, 1H, H-4 of cyclopentanol), 1.79–1.88 (m, 1H, H-3 of cyclopentanol), 1.95–2.06 (m, 3H, H-2, H-3, H-4 of cyclopentanol), 2.81 (s, 1H, OH), 4.12 (s, 1H, OH), 4.46 (s, 1H, CH(OH)-phenyl), 4.47 (d, 1H, J=14.2 Hz, CH_2-triazolyl), 4.66 (d, 1H, J =14.2 Hz, CH_2-triazolyl), 5.73 (s, 1H, OH), 7.21 (d, 2H, J =8.25 Hz, H-aromatic), 7.28 (d, 2H, J=8.25 Hz, H-aromatic), 7.84 (s, 1H, H-triazole), 8.28 (s, 1H, H-triazole); MS, M^+ 365. The metabolite AM3 was identified as (1*RS*,-2*RS*,5*RS*)-5-(4-chlorobenzyl)-2-(1-hydroxy-1-methylethyl)-3-hydroxy-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol (Fig. 2), based on the following evidence: ^{1}H NMR (250 MHz, $CDCl_3$) δppm, 1.51 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.62–1.68 (m, 2H, H-4 of cyclopentanol), 1.79 (d, 1H, J =4.9 Hz, H-2 of cyclopentanol), 2.14–2.21 (m, 1H, H-5 of cyclopentanol), 2.43 (dd,1H, J=5.5 Hz, 13.4 Hz, CH_2-phenyl), 2.59 (dd,1H, J=11.0 Hz, 13.4 Hz, CH_2-phenyl), 3.42 (d, 1H, J=3.1 Hz, OH), 4.13 (s, 1H, OH), 4.26 (d, 1H, J=14.0 Hz, CH_2-triazolyl), 4.56 (d, 1H, J=14.0 Hz, CH_2-triazoly), 4.56 (m, 1H, H-3 of cyclopentanol, 5.16 (s, 1H, OH), 6.94 (d, 2H, J=8.5 Hz, H-aromatic), 7.19 (d, 2H, J =8.5 Hz, H-aromatic), 7.95 (s, 1H, H-triazole), 8.17 (s, 1H, H-triazole); MS, M^+ 365. The metabolite AM4 was identified as (1*RS*,2*SR*,5*RS*)-5-(4-chlorobenzyl)-3-hydroxy-2-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol (Fig. 2), based on the following evidence: ^{1}H NMR (500 MHz, $CDCl_3$, D_2O) δppm, 1.14 (d, 3H, J=6.9 Hz, CH_3), 1.21 (d, 3H, J=6.4 Hz, CH_3), 1.38 (dd, 1H, J=3.2 Hz, 9.2 Hz, H-2 of cyclopentanol), 1.45 (dd, 1H, J=4.1 Hz, 14.7 Hz, H-4 of cyclopentanol), 1.73–1.79 (m, 1H, H-4 of

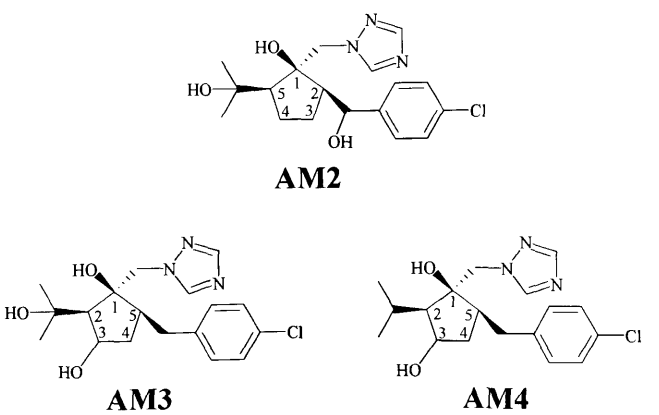

Fig. 2. Chemical structures of the metabolites of ipconazole isolated from the culture of *Streptomyces* sp. D16.

cyclopentanol), 1.81 (dd, 1H, J=3.7 Hz, 10.8 Hz, CH_2-phenyl), 2.22–2.32 (m, 2H, C<u>H</u>$(CH_3)_2$, CH_2-phenyl), 2.41–2.46 (m, 1H, H-5 of cyclopentanol), 4.23 (d, 1H, J=14.2 Hz, CH_2-triazolyl), 4.35 (m, 1H, H-3 of cyclopentanol), 4.62 (d, 2H, J=14.2 Hz, CH_2-triazolyl), 6.98 (d, 2H, J=8.3 Hz, H-aromatic), 7.19 (d, 2H, J=8.3 Hz, H-aromatic), 7.97 (s, 1H, H-triazole), 8.21 (s, 1H, H-triazole); MS, M^+ 349.

Because of difficulty in monitoring the polar metabolites in the fractions of chloroform/methanol (90/10) and methanol on HPLC with a UV detector, further work on these fractions was not attempted.

4. Metabolic Study of Isolates A1 and D16 Using ^{14}C-Ipconazole

The depletion of ^{14}C-ipconazole and its metabolites by *Kitasatospora* sp. A1 and *Streptomyces* sp. D16 is shown in Tables 4 and 5, respectively. *Kitasatospora* sp. A1 had degraded more than 80% of the ipconazole after a 3-day incubation. The primary metabolites, M1 and M12, accounted for 8.1% and 6.4%, respectively, of the applied ipconazole. The metabolites AM2, M5, M6, and M13 were also detected, but in amounts less than 3%. The metabolite M22 (1,2,4-triazole) was not detected in the culture after 1 day of incubation, but accounted for 3.9% of the applied ipconazole after 3 days. Volatized compounds and carbon dioxide were not found. *Streptomyces* sp. D16 had degraded approximately 20% of the ipconazole after 2 days, but more than 99% after 6 days of incubation. The metabolites detected from the culture of isolate A1 were also formed in the culture of the isolate D16, while only a trace amount of AM4 was detected in the D16 culture. The metabolite of AM3 was not found in the experiment using ^{14}C-ipconazole. The polar metabolites in the ethyl acetate fraction had increased by the end of the incubation in both the A1 and D16 culture. Representative HPLC chromatograms of the ethyl acetate fraction from the D16 culture are shown in Fig. 3.

DISCUSSION

There have been a few studies of the biodegradation or depletion of triazole fungicides in soil. Concerning the microbial degradation of triazole fungicides, the metabolism of diniconazole to 1,2,4-triazole or 2,4-dichlorobenzoic acid by diniconazole-degrading microorganisms in soil has been reported.[4] The number of ipconazole degraders was greatest in actinomycetes, followed by fungi. Only one isolate showed ipconazole-degrading activity in the 39 bacterial strains tested. Microbial degradation does appear to be affected by nutritional conditions in some cases.[17] As such, the use of the nutrient broth for bacterial strains may explain in part the lack of bacteria having ipconazole-degrading activity.

Two ipconazole degraders in the actinomycetous isolates showing strong degrading activity were identified as *Kitasatospora* sp. and *Streptomyces* sp. They decomposed 1 μg/ml of ipconazole rapidly in the presence of an available carbon source, but their population did not grow in the mineral salt medium containing ipconazole as a carbon source (data not shown). These findings suggest that these actinomycetes degrade ipconazole through a co-metabolic

Table 4. Degradation of ^{14}C-ipconazole by *Kitasatospora* sp. A1

Fraction	Percentage of applied radioactivity [a]		
	0 days	1 days	3 days
Ethyl acetate fraction	95.8	98.4	95.6
AM2	n.d. [b]	n.d.	1.9
AM4	n.d.	n.d.	n.d.
M1	n.d.	1.2	8.1
M5	n.d.	0.1	1.2
M6	n.d.	n.d.	1.1
M12	n.d.	1.7	6.4
M13	n.d.	n.d.	2.3
ipconazole	93.9	92.8	18.7
others	1.9	2.6	55.9
Water-soluble fraction	0.1	0.2	4.2
M22	n.d.	n.d.	3.9
Residue	0.5	0.4	0.2
CO_2	- [c]	n.d.	n.d.
Total	96.4	99.0	100

[a] 65.8 kBq in a petri dish. [b] Not detected. [c] Not analyzed.

Table 5. Degradation of ^{14}C-ipconazole by *Streptomyces* sp. D 16

Fraction	Percentage of applied radioactivity [a]		
	0 days	2 days	6 days
Ethyl acetate fraction	93.3	100.4	95.2
AM2	n.d. [b]	n.d.	2.6
AM4	n.d.	0.5	n.d.
M1	n.d.	5.0	n.d.
M5	n.d.	1.5	1.0
M6	n.d.	1.2	0.4
M12	n.d.	5.0	5.0
M13	n.d.	0.8	1.1
ipconazole	91.2	79.4	0.8
others	2.1	7.0	84.3
Water-soluble fraction	1.4	0.6	5.6
M22	n.d.	n.d.	5.1
Residue	0.2	0.1	0.3
CO_2	- [c]	n.d.	n.d.
Total	94.9	101.1	101.1

[a] 62.3 kBq in a petri dish. [b] Not detected. [c] Not analyzed.

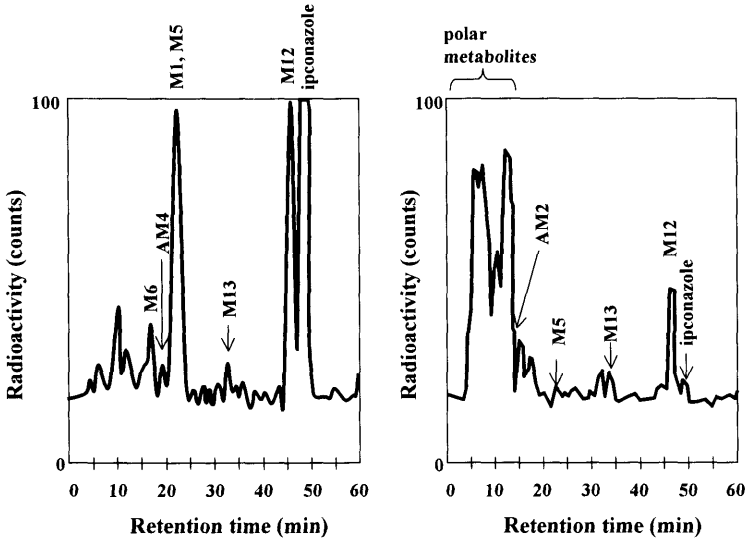

Fig. 3. HPLC chromatograms of the metabolites of ^{14}C-ipconazole in the ethyl acetate fraction from the culture of *Streptomyces* sp. D16 after a 2-day incubation (left) and after a 6-day incubation (right).
Column: Inertsil ODS-2, 4.6 mm×250 mm (GL Science). Flow rate: 0.8 ml/min. Solvent system: CH_3CN/H_2O (45/55) up to 34 min, and CH_3CN/H_2O (75/25) from 34 min to 60 min. Detection: radioactivity (RLC-551, Aloka).

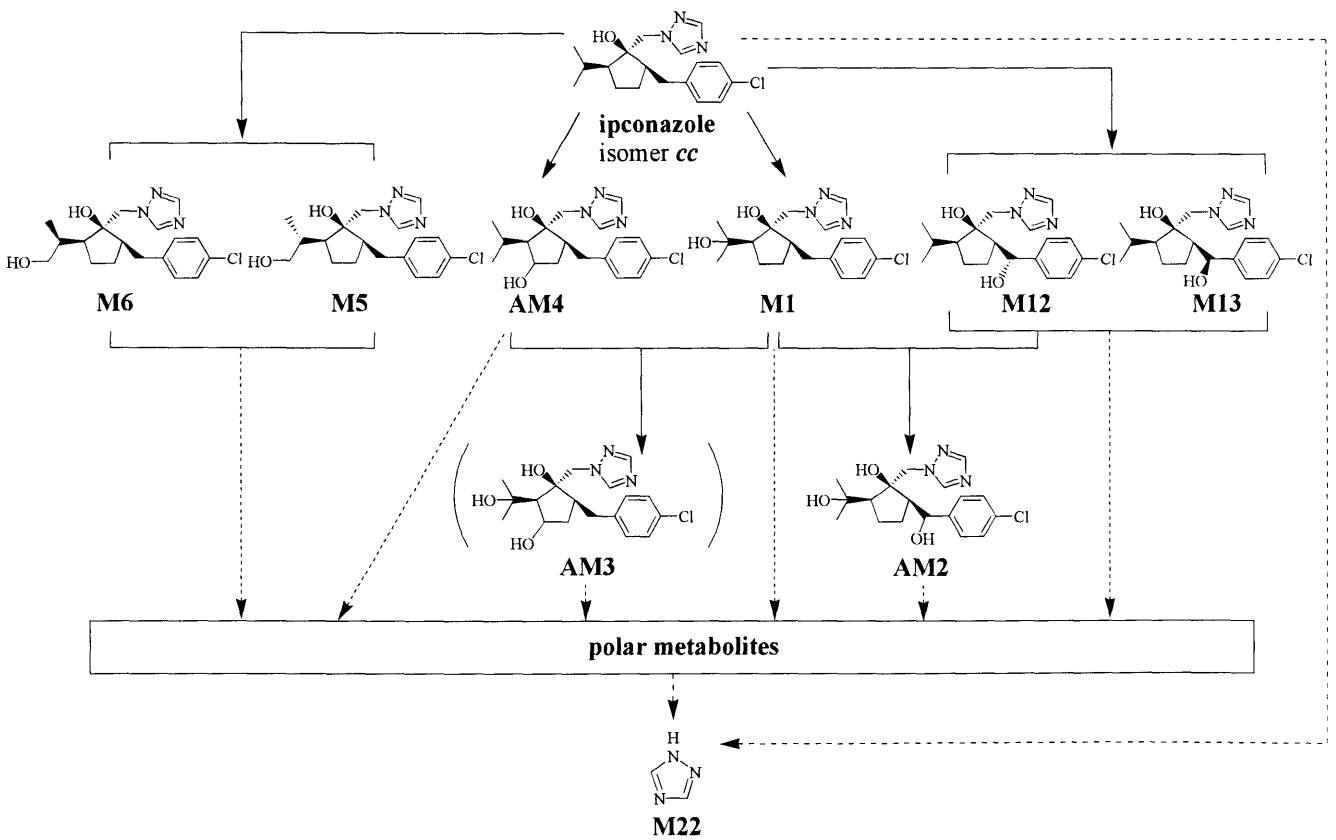

Fig. 4. The probable metabolic pathways for ipconazole degraded by the actinomycetous isolates A1 and D16.

pathway. The probable metabolic pathways of the actinomycetous isolates A1 and D16 are summarized in Fig. 4. The main metabolites were found to be M1, M12, and M22, with M5, M6, M13, AM2, AM3, and AM4 detected as minor metabolites. Therefore, the primary metabolic reaction could be dominated by hydroxylation at the carbons of the methine in the isopropyl group and the benzylmethylene. *Kitasatospora* sp. A1 and *Streptomyces* sp. D16 also

possess oxidation ability at either the carbon of the methyl portion in the isopropyl group or that of the methylene portion of the cyclopentane ring. The polar metabolites in ethyl acetate extracts had increased by the end of the incubation (Tables 4 and 5). These metabolites were not identified, but the formation of the dihydroxylated metabolite AM2 or AM3 led to the formation of another multi-hydroxylated ipconazole. The oxidation of the primary or secondary alcohol to carboxyl groups or ketones may be included in the degradation process. A variety of diastereomers of ipconazole metabolites might be produced by oxidation at the carbon of the methyl portion in the isopropyl group, that of the methylene portion of the cyclopentane ring, and that of the benzylmethylene. The formation ratio of diastereomer (M12/M13) suggests that stereoselective hydroxylation at the benzylmethylene may occur. Regarding the degradation of diniconazole by soil microorganisms, the formation of triazole, M22, had been reported.[4] M22 was also detected in the water-soluble fraction from the culture of isolated actinomycetes exposed to ^{14}C-ipconazole. M22 could be generated from ipconazole or its metabolites by oxidation at the carbon of the triazolyl methylene (Fig. 4). The metabolites of ipconazole converted by the A1 and D16 isolates were similar to those found in rice seedlings.[1] But dihydroxylated metabolites like AM2 and AM3, and monohydroxylated metabolites at the alicyclic carbon like AM4 have not been found in rice plants. Although there is little information available regarding the microbial degradation of azole compounds, strains of bacteria, actinomycetes, and fungi having the ability to degrade ipconazole, were isolated from the soil. Among the isolates, some actinomycetes showed high levels of degrading activity for ipconazole in the liquid medium. This finding suggests that some soil microorganisms contribute to the biodegradation of a triazole fungicide like ipconazole in the soil.

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