Anaerobic Microorganisms Degrading 3-Methylindole (Skatole) and Indole in Composting Processes

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Abstract Anaerobic microorganisms that degrade indole or 3-methylindole (skatole) were isolated from porcine or chicken manure composting processes. Numbers of indole or skatole-degrading microorganisms in the pretreatment stage and the finishing stage (mesophilic stages) were larger than those in the fermentation stage (thermophilic stage). Thermophilic anaerobes degrading these compounds were not detected at any stage. Ninety-two isolates were classified into 4 types, *i.e.* facultatively anaerobic gram-positive cocci, facultatively anaerobic gram-positive rods, obligately anaerobic gram-positive endspore-forming rods and facultatively gram-positive endspore-forming rods. Among them, 67 to 91% were facultatively anaerobic. Utilization of indole or skatole by 24 isolates were quantitatively investigated. All of these isolates degraded indole, but only 8 isolates degraded skatole. Three of skatole-degrading isolates were obligate anaerobic bacteria. This is the first report which indicate the existence of such a type of obligate anaerobes.

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Key words : Indole, Skatole, Compost, Anaerobic microorganisms

Compost production processes have been widely used as a waste treatment system. Composting has advantages in stabilizing large volumes of wastes, disinfection of pathogenic organisms, production of fertilizer or soil conditioners. For these reasons, composting possesses a definite and useful role in the implementation of sustainable agriculture²⁶⁾.

Indole and its derivatives such as 3-methylindole (skatole) form a class of toxic recalcitrant N-heterocyclic compounds. They become pollutants after released from cigarette smoke^{8,10,17)}, Coal tar²⁵⁾, and sewage²⁵⁾ to environment. Although the general level of indoles in living environment is less than the level of immediate toxicity, higher level of

skatole cause acute pulmonary edema, emphysema, lung diseases⁷⁾, hemoglobinuria, and hemolysis¹²⁾ in cattle and goats. Skatole and indole are main components of the stench of faeces and are resistant to degradation^{3,6)}. They are the main cause of unpleasant smell of agricultural wastes. The destruction of malodorous compounds is one of the most important problems for animal wastes treatment. Since the number of anaerobic microorganisms was one tenth of the number of aerobic microorganisms in composting processes²⁴⁾ and anaerobic condition prevail in interior of composts under some circumstances^{20,27)}, it is important to examine the anaerobic biodegradation potential of these compounds. In this study, we determined the number of these

compounds degrading bacteria in compost production process. We also determined the ability of the isolates to degrade these compounds.

Materials and Methods

Sample

Samples were collected from compost plants of porcine wastes (scoop type) and poultry wastes (windrow type) at Miyagi Prefecture, Japan. A term of composting was about 6 months in porcine wastes treatment system and about one month in poultry wastes treatment system. About 500 g of samples were obtained from 30 cm depth at three stages of composting, *i.e.* the pretreatment stage, the fermentation stage and the finishing stage, of which temperature was 40, 75 and 20°C in the porcine waste treatment system and 50, 60 and 20°C in the poultry waste treatment system, respectively. Moisture of samples were 60, 65 and 25% in respective stage in porcine waste treatment system, and 50, 60 and 20% in poultry waste treatment system. At pretreatment stage, straw was added to the manure as an amendment in porcine waste treatment system and wood chip was added in poultry waste treatment system. Compost recycle was used along with amendment addition in both systems.

Bacterial count

Anaerobic bacteria were counted by 10-fold dilution methods using a sterilized dilution solution⁵⁾ and cultivation by the roll tube method¹⁶⁾ modified by Bryant⁴⁾. The standard tube count was performed with M2 medium¹⁴⁾ added with the compost extracted solution instead of the rumen fluid. Compost extracted solution was prepared as described by Gordon and Rynearson⁹⁾. Numbers of indole and skatole-degrading anaerobic microbes were determined by colony counts on media which contained indole or skatole as a sole source of carbon. The media were modified Hobson's medium¹⁴⁾ containing the following nutrients; indole or skatole, 0.3 g, NaCl, 0.9 g, KH₂PO₄, 0.45

g, (NH₄)₂SO₄, 0.45 g, MgSO₄, 0.09 g, CaCl₂, 0.09 g, K₂HPO₄, 0.45 g, 0.1% resazurin solution, 1 ml, L-cystein hydrochloride monohydrate, 0.5 g, agar, 18 g, pH 7.5 in 1 l of water. Indole and skatole were dissolved in a minimum amount of methanol and added to the autoclaved medium. As a control set, we prepared the media which contained the equal volume of methanol without indole or skatole. The anaerobic cultures were incubated 14 days at room temperature, 37° or 56°C.

The ability of the isolates to degrade indole and skatole

The isolated strains were incubated in PYG medium¹⁵⁾ added with 0.01% indole or skatole for 7 days at 37°C. Degradability of the substrates was determined by the rate of disappearance of the substrates. All experiments were conducted in triplicate.

Gas chromatography system and column

Analyses were performed by Shimadzu gas chromatograph (Model GC-12 A) and Shimadzu integrator (Chromatopac C-R 5 A). A 30-m fused-silica capillary column ULBON HR-1701 (Shinwa chemical industries, Ltd., Kyoto, Japan), coated with polyimide resin with a film thickness of 0.53 µm, was used. The carrier gas was nitrogen at a column flow-rate of 0.1 kg/cm². The injector and detector temperatures were set to 300°C. The temperature gradient was from 120 to 250°C at 10°C/min. Total analysis time was normally 25 min, including the time for cooling the oven.

Indole and skatole extraction procedure was carried out as described by Jensen *et al.*¹⁸⁾ with minor modifications.

Results

Indole and skatole-degrading anaerobic microbial flora of composting system

Tables 1 and 2 showed numbers of anaerobic bacteria in compost processes. At the porcine waste treatment system, the level of mesophilic standard tube count decreased after pretreatment stage and increased after fermentation

Anaerobic Biodegradation of Skatole in Compost

Table 1. Distribution of numbers of anaerobic microbes in composting processes

Microbial types	Cultivation at room temp. (25–30°C)			Cultivation at 37°C		
	Pretreatment stage	Fermentation stage	Finishing stage	Pretreatment stage	Fermentation stage	Finishing stage
Porcine waste						
STC ^{a)}	7.60±0.15 ^{b)}	4.90±0.16	5.24±0.16	7.93±0.04	4.95±0.05	7.18±0.10
IDM	3.51±0.10	2.16±0.28	3.03±0.31	3.26±0.40	2.20±0.17	2.97±0.14
SDM	2.86±0.56	<2.00	2.58±0.27	2.99±0.46	<2.00	3.60±0.16
Poultry waste						
STC	7.01±0.08	5.46±0.15	5.93±0.06	7.76±0.12	6.21±0.19	6.88±0.21
IDM	3.17±0.19	2.23±0.40	3.16±0.10	3.42±0.07	2.10±0.17	3.18±0.20
SDM	2.26±0.24	2.10±0.17	3.00±0.37	2.49±0.20	<2.00	3.29±0.23

^{a)} Microbial types : STC, standard tube count ; IDM, indole-degrading microbe ; SDM, skatole-degrading microbe.

^{b)} Data are expressed as mean of log₁₀ CFU/g wet weight±standard deviation.

Table 2. Distribution of numbers of thermophilic anaerobes in composting processes

Microbial types	Cultivation at 56°C		
	Pretreatment stage	Fermentation stage	Finishing stage
Porcine waste			
STC ^{a)}	5.12±0.14 ^{b)}	4.23±0.04	2.89
IDM	<2.00	<2.00	<2.00
SDM	<2.00	<2.00	<2.00
Poultry waste			
STC	4.01±0.08	2.46±0.15	2.04
IDM	<2.00	<2.00	<2.00
SDM	<2.00	<2.00	<2.00

^{a)} Microbial types : STC, standard tube count ; IDM, indole-degrading microbe ; SDM, skatole-degrading microbe.

^{b)} Data are expressed as log₁₀ mean CFU/g wet weight±standard deviation.

stage. On the other hand, thermophilic standard tube counts decreased as the treatment progressed. Standard tube counts at 37°C cultivation were higher than those at room temperature or 56°C. Similar pattern was observed in the poultry waste treatment system.

At the porcine waste treatment system, the change of levels of mesophilic indole-degrading microbes was similar to that of mesophilic standard tube counts. The ratio of indole-degrading microbes to standard tube count

ranged from 0.01 to 0.001% in all stages. Mesophilic indole-degrading microbes were not different in number between 37°C and room temperature cultivation. Thermophilic indole-degrading microbes were not detected at any processes. Similar results were obtained at the poultry wastes composting processes. The change of numbers of mesophilic skatole-degrading microbes was similar to that of mesophilic indole-degrading microbes. Mesophilic skatole-degrading microbes were slightly lower in number than indole-degrading

microbes. Thermophilic skatole-degrading microbes were not detected at any process. Similar results were obtained at poultry waste treatment system.

Distribution of bacteria degrading these compounds was investigated among isolates. In the compost plant of porcine waste, four

types of bacteria, facultatively anaerobic gram-positive cocci, facultatively anaerobic gram-positive rods, obligately anaerobic gram-positive endospore-forming rods and facultatively gram-positive endospore-forming rods, were isolated (Table 3). Neither of gram-negative bacteria, actinomyces nor fungi

Table 3. Types of indole and skatole-degrading anaerobic microbes isolated from the compost process of porcine waste

Microbial flora	Pretreatment stage		Fermentation stage		Finishing stage	
	No. of isolates	% ^{a)}	No. of isolates	%	No. of isolates	%
Indole-degrading bacteria						
FPC ^{b)}	8	29.6	3	20.0	2	13.3
FPR	5	18.5	4	26.7	5	33.3
OPER	10	37.0	7	46.7	5	33.3
FPER	4	14.8	1	6.7	3	20.0
Skatole-degrading bacteria						
FPC	6	20.0	—	—	7	28.0
FPR	16	53.3	—	—	13	52.0
OPER	8	26.7	—	—	5	20.0
FPER	0	0.0	—	—	0	0.0

^{a)} Percentage of total isolates.

^{b)} Microbial flora: FPC, facultatively anaerobic gram-positive cocci; FPR, facultatively anaerobic gram-positive rods; OPER, obligately anaerobic gram-positive endospore-forming rods; FPER, facultatively anaerobic gram-positive endospore-forming rods.

Table 4. Types of indole and skatole-degrading anaerobic microbes isolated from the compost process of poultry waste

Microbial flora	Pretreatment stage		Fermentation stage		Finishing stage	
	No. of isolates	% ^{a)}	No. of isolates	%	No. of isolates	%
Indole-degrading bacteria						
FPC ^{b)}	7	33.3	5	41.7	4	23.5
FPR	6	28.6	5	41.7	6	35.3
OPER	5	23.8	2	16.6	5	29.4
FPER	3	14.3	0	0.0	2	11.8
Skatole-degrading bacteria						
FPC	5	35.7	—	—	8	38.1
FPR	4	28.6	—	—	7	33.3
OPER	2	14.3	—	—	2	9.5
FPER	3	21.4	—	—	4	19.0

^{a)} Percentage of total isolates.

^{b)} Microbial flora: FPC, facultatively anaerobic gram-positive cocci; FPR, facultatively anaerobic gram-positive rods; OPER, obligately anaerobic gram-positive endospore-forming rods; FPER, facultatively anaerobic gram-positive endospore-forming rods.

were detected at any stage. The dominant indole-degrading bacteria was obligately anaerobic gram-positive endspore-forming rods in all stages. The dominant skatole-degrading bacteria was facultatively anaerobic gram-positive rods. Facultatively anaerobic gram-positive endspore-forming rods were not isolated among skatole-degrading bacteria.

In the compost plant of poultry wastes, the same types of bacteria were investigated (Table 4). The dominant indole-degrading bacteria was facultatively anaerobic gram-positive cocci in the pretreatment and fermentation stages and was facultatively anaerobic gram-positive rods in fermentation and finishing stages. The dominant skatole-degrading bacteria was facultatively anaerobic gram-positive cocci. Obligately anaerobic gram-positive endspore-forming rods were the smallest among skatole-degrading bacteria in number.

The ability of the isolates to degrade indole and skatole

All of 24 isolates degraded indole and 8 isolates degraded skatole (Table 5). The largest rate of disappearance of indole (28.64%) was shown by facultatively gram-positive endspore-forming rods HA 2-4 and smallest (3.30%) was shown by obligately anaerobic gram-positive endspore-forming rods IEI-14. On the other hand, the largest rate of disappearance of skatole (11.91%) was shown by obligately anaerobic gram-positive endspore-forming rods A-3 and smallest (3.64%) was shown by obligately anaerobic gram-positive endspore-forming rods A-4.

Discussion

Indole or skatole-degrading bacteria appeared during the mesophilic stage, and their numbers decreased in the thermophilic stages. After the temperature lowered, they reappeared. This pattern was commonly investigated for mesophilic bacteria in composts²⁴). A similar pattern was investigated in standard tube counts in this ex-

Table 5. Utilization of indole and skatole by the isolated strains

Strain	Rates of disappearance (%)	
	Indole	Skatole
FPC ^{a)}		
A-1	22.23	0.00
A 1-3	26.19	0.00
HA 2-1	17.05	0.00
HA 2-3	20.26	3.93
S 2-10	27.36	11.69
FPR		
A 1-2	11.99	8.09
HA 2-2	23.48	0.00
S 2-11	27.94	10.64
OPER		
A-3	16.53	11.91
A-4	6.21	3.64
A-13	11.06	0.00
A 1-4	24.89	0.00
A 1-5	10.73	0.00
A 1-6	16.12	4.76
IEI-2	11.38	0.00
IEI-5	8.19	0.00
IEI-14	3.30	0.00
I 21-5	8.79	0.00
I 21-7	23.23	0.00
FPER		
HA 2-4	28.64	0.00
HA 2-5	11.38	0.00
I 21-13	15.88	0.00
I 21-14	27.05	0.00
I 31-1	8.71	10.14

^{a)} Microbial type : FPC, facultatively anaerobic gram-positive cocci ; FPR, facultatively anaerobic gram-positive rods ; OPER, obligately anaerobic gram-positive endspore-forming rods ; FPER, facultatively anaerobic gram-positive endspore-forming rods.

periment. Skatole-degrading microbes were slightly lower in number than indole-degrading microbes. This result agrees with the observation that not all indole-degrading isolates were capable of degrading skatole but skatole-degrading isolates were all capable of degrading indole (Table 5). There is a possibility that mesophilic microbes were replaced by

thermophilic microbes during thermophilic stage. Then, we attempted to determine the numbers of thermophilic indole or skatole-degrading microbes. When cultivation temperature was set to 56°C, they were not detected at any stages (Table 2). In addition to such experiments, we also carried out the enrichment culture of thermophilic indole and skatole-degrading microbes and did not isolate them (data not shown). These results indicated that few thermophilic anaerobic skatole and indole-degrading microbes were present in the compost. A striking difference was not recognized between plants of porcine wastes and poultry wastes. Kamath and Vaidyanathan¹⁹⁾ reported *Aspergillus niger* which did not grow when indole was used as sole source of carbon and nitrogen but cometabolized indole when glucose and nitrate were present in the medium. Since such microbes could not be grow on the medium used in the present work, numbers of indole or skatole-degrading microbes may higher than the present results.

Almost all isolates degrading these compounds were facultative anaerobes (Tables 3 and 4). Strict aerobic or facultatively anaerobic microbes were known to degrade indole or skatole under aerobic condition²³⁾. The present study indicated that many types of bacteria were capable of degrading indole or skatole also under anaerobic condition. Anaerobic degradation of indole shown by cultivation of municipal sewage sludges or after enrichment cultures^{2, 11, 12, 21, 22)}. *Desulfobacterium indolicum*, a sulfate-reducing bacterium, which was isolated from marine sediment, was the definite species to be shown to use indole as the sole electron donor and carbon source¹⁾. The present paper is the first report which showed that obligate anaerobes could anaerobically degrade skatole. We are clarifying the pathway for the biodegradation of indole or skatole by the isolates. The information should be useful in determining the fate of these chemicals in animal manure treatment

systems.

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コンポスト処理過程における 3-メチルインドール（スカトール） またはインドールを分解する嫌気性菌の動態解析

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ブタ排泄物およびニワトリ排泄物のコンポスト形成過程から難分解性の悪臭物質インドールおよび 3-メチルインドール（スカトール）を嫌気的に分解する微生物の動態解析を行った。インドール分解菌、スカトール分解菌ともに発酵過程（高温期）よりも前処理過程（中温期）および製品コンポストに菌数が多いことが示された。また、好熱性のインドールもしくはスカトール分解菌はいずれの処理過程からも検出されなかった。これらの化合物の分解菌を 92 株分離したところ、通性嫌気性グラム陽性球菌、通性嫌気性グラム陽性桿菌、通性嫌気性グラム陽性芽胞桿菌、偏性嫌気性グラム陽性芽胞桿菌の 4 タイプの菌群に分類された。分離株の大部分（67-91%）は通性嫌気性菌であった。分離株はすべてインドール分解能を有していたが、スカトール分解能を有する株は 8 株のみであった。8 株のスカトール分解菌の中で 3 株が偏性嫌気性菌であり、これはスカトール分解能を有する偏性嫌気性菌の存在を初めて明らかにするものである。

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