Analysis of Malodorous Substances of Human Feces

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Various malodorous substances generated from human feces were analyzed immediately after the use of a Western-style toilet by 50 subjects. The types and amounts of these malodorous components varied slightly between individuals, depending on the food that they had eaten and their state of health. Hydrogen sulfide was detected at concentrations of 5–26 ppb, methyl mercaptan at 2–15 ppb, ammonia at less than 100 ppb, propylaldehyde, fatty acids, and pyridine at about 10 ppb, and trimethylamine at around trace levels. When subjects had diarrhea, the amounts of fatty acids, particularly acetic acid, in feces were more than 100000-fold higher than in feces of those in normal health.

Key words —— human fece, malodorous substance, state of health

INTRODUCTION

The toilet is typically a source of offensive odors in dwellings.[1,2] Those offensive odors are a mixture of the odor of excreted feces itself and that of raw sewage generated by the decomposition of feces. However, the odor from a flush-type toilet is primarily due to the odor of excretion. The type of malodorous components is thought to be influenced by many factors such as age, diet, health status, and the use of drugs.

Various methods, including adsorption by activated carbon, oxidization using catalysts, and ozone decomposition, have been developed to eliminate the offensive odor of feces. One effective method is microbial deodorization. Compared with the other methods mentioned, the merits of microbial deodorization are its specificity in decomposing malodorous substances, low cost, and the ease of maintenance of the deodorizing system.

We previously reported the isolation of certain bacteria from the soil[3,4] which can decompose hydrogen sulfide and methyl mercaptan and have potential applications in microbial deodorization. Generally, the odor of feces is attributed to fatty acids, sulfur-containing compounds, indole, skatole, and ammonia.[5–9] However, systematic analysis of the malodorous components has not been conducted. Therefore we conducted such an analysis of the malodorous components immediately after the use of a flush toilet with a view to using the bacteria previously isolated for microbial deodorization. Thermal-desorption cold-trap injector/gas chromatography/mass spectrometry (TCT/GC/MS) measurements were performed to identify the malodorous compounds.

MATERIALS AND METHODS

Chemicals —— A commercially available adsorbent agent, Tenax-TA (GL Sciences Inc., Japan), was used to collect gas samples. Standard reagents were purchased from Wako Pure Chemical Industries (Japan) and Tokyo Kasei Kogyo Co. (Japan). All other chemicals used were commercially available and of chemically pure grade. Distilled water, which was purified with a Milli-QSP system (Millipore, Milford, MA, U.S.A.), was used for the preparation of all aqueous solutions.

Collecting Malodorous Substances during Defecation —— The apparatus shown in Fig. 1 was placed in the toilet [room size 97 cm (D) × 127 cm (W) × 185 cm (H)] in a laboratory, and gas samples from the toilet during defecation by an adult male were collected in a 100-l sampling bag. A Teflon flexible joint was used at the junction (71 mm in internal diameter, 105 mm in external diameter) between the Tedlar sampling bag and the small pump. The Tedlar bag was suspended during sampling to minimize the effect of pressure loss. The toilet was deodorized before sampling using a small deodorizer (Minifume Clean Filter, containing activated carbon fibers, Toyobo, Japan) to reduce the background reading. The malodorous substances of fe-

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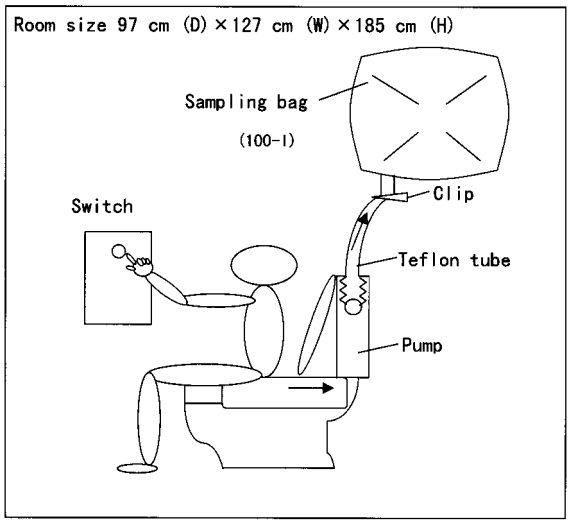

Fig. 1. Method of Sampling Malodorous Compounds from Human Feces in a Toilet

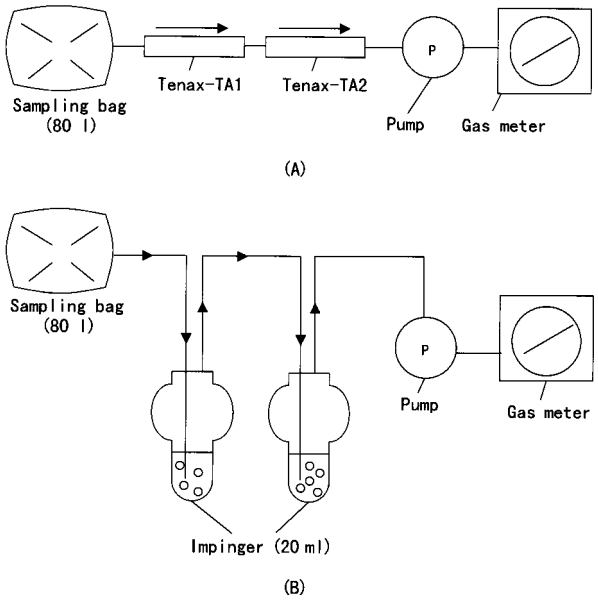

Fig. 2. Two Types of the Apparatus to Collect Malodorous Compounds in Human Feces

(A): For fatty acids, aliphatic and aromatic compounds. (B): For ammonia and amines.

ces were collected as follows: (1) aspirating the air from the toilet at the rate of 150 l/min by turning on the pump switch immediately after defecation; (2) turning off the switch after 30 sec; and (3) sealing the Tedlar bag with a large clip. About 80 l of gas was aspirated. The volume of the aspirated malodorous gas was measured using a wet gas meter (W-NK-1B, Shinagawa Keisokuki, Japan). Of the 50 samples analyzed in this study, the volume of gas aspirated for 47 samples was 80 l and 40 l for the remaining three samples.

Identification of Malodorous Substances of Human Feces —— In this study, 10 l of the sample gas in the Tedlar bag was aspirated using a pump into a condensation tube packed with the trapping agent Tenax-TA (200 mg). The tube was 5 mm in external diameter (3 mm in internal diameter) and 250 mm long, onto which the malodorous substances were adsorbed. Simultaneous qualitative analysis of all the malodorous substances of feces was performed by treating the contents of this condensation tube using the TCT/GC/MS technique.

Next, we carried out quantitative analysis of fatty acids, aromatic hydrocarbon compounds, sulfur-containing compounds, and nitrogen-based compounds (*e.g.*, ammonia and amines), which are considered to be the primary components of the odor of excretion. Mass spectral identifications and quantitative measurements were performed by methods similar to those used in our previous study.[10]

Determination of Fatty Acids, and Aliphatic and Aromatic Compounds —— After 10 l of the malodorous components in the sampling bag were adsorbed at room temperature onto the trapping agent (Tenax-TA) by the method shown in Fig. 2(A), the volatile compounds on the trapping agent were analyzed using TCT/GC/MS under the conditions shown in Table 1. A polar DB-WAX analysis column (J&W Scientific, Folsom, CA, U.S.A.) was used, which can detect all of the target substances, such as short-chain fatty acids, sulfur-containing compounds, pyridine, and pyrrole, with a high degree of reproducibility.

Determination of Concentration of Sulfur-Containing Compounds —— Volatile sulfur-containing compounds were analyzed by GC using a Flame photometric detector(FPD) as a detector after cold trapping of the gas in a Tedlar bag. The gas collected in the bag shown in Fig. 1 was aspirated (0.5–2.0 l) into the cold-trapping tube and was injected into the gas chromatograph and analyzed under the conditions listed in Table 2 according to the analytical methods for offensive odors specified by Japan Industrial Standards (JIS) K0092[11] and K0108.[12] Hydrogen sulfide and methyl mercaptan at a concentration of 100 ppm in nitrogen were used as standard gases for the preparation of our calibration curves.

Determination of Ammonia Concentration —— The ammonia assay was performed by the method recommended for offensive odor analysis using JIS K 0099.[13] The concentrations of malodorous com-

Table 1. Conditions for Determining the Concentration of Fatty Acids, and Aliphatic and Aromatic Compounds

TCT	Chrompack
Sample injection method	Thermal heating desorption and reconcentration
Trap	DB-1 (J & W), i.d. 0.25 mm × 0.3 m, film thickness 0.25 μm
Trap temperature	−130°C
Desorption tempertature	240°C
Desorption time	10 min
Desorption frow rate	10 ml/min
Injection temperature	200°C
Injection time	3 min
GC/MS	Hewlett Packard HP5890 + 5970B
Column	DB-WAX (J & W), i.d. 0.25 mm × 30 m, film thickness 0.5 μm
Column temperature	16 min at 30°C, 5°C/min to 250°C
Injection temperature	200°C
Carrier gas	He, 1 ml/min
Separator temperature	280°C
Detector	MS; Scan range (m/z 10–500), selected ion monitoring

TCT, thermal-desorption cold trap injector.

Table 2. Conditions for Determining Concentration of Sulfur Compounds

Gas chromatograph	Hewlett Packard HP5890II
Column	β,β'-oxydipropionitrile (GL Sciences Inc.)
	Glass i.d. 2 mm × 2.5 m
Column temperature	70°C (isothermal)
Injection temperature	200°C
Carrier gas	He, 30 ml/min
Detector	Flame photometric detector(FPD), 200°C

ponents were determined as follows. The gas collected in the Tedlar bag was aspirated at a rate of 0.5 l/min into the sample collection apparatus containing 0.01 M H_2SO_4 for 40 min. After aspiration of the gas, the trapping solutions contained in two absorption bottles were then pooled and transferred to a 50-ml graduated flask. The interior of the absorption bottles was washed with the trapping solution, and this wash fluid was mixed with the trapping solution in the graduated flask. Ten milliliters of this solution was placed in a stoppered test tube and used as a sample for analysis.

The absorbance of the samples was measured as follows. After adding 5 ml of 1% phenol/0.005% sodium pentacyano-nitrosylferrate (III) solution to each sample and mixing well by shaking, 5 ml of 0.05% sodium hypochlorite solution was added. A stopper was applied, and the contents were mixed gently. The mixture was allowed to stand for 1 hr at 25–30°C, and the absorbance at 640 nm was measured. A sample of the trapping solution that had not been bubbled with the air sample was treated in a similar way and served as a control.

Determination of Concentration of Amines and Aldehydes —— The sample gas collected in the sampling bag was bubbled through an aqueous solution of 0.01 M H_2SO_4 for 40 min at a rate of 0.5 l/min, as shown in Fig. 2B. The pH of this gas-trapping solution was adjusted to about 7 with 1 M NaOH, and the solution was then passed through a Millipore filter (pore size, 0.44 μm). The amine concentration was subsequently determined by ion chromatography under the conditions shown in Table 3.

The aldehydes was determined based on the Offensive Odor Control Law.[14)]

RESULTS AND DISCUSSION

Evaluation of Breakthrough Volume of Malodorous Components in Tenax-TA

Leakage of malodorous compounds from the adsorbent is known as breakthrough, and the volume of gas that has passed through at that point is

Table 3. Conditions for Determining Concentrations of Amines

Ion chromatography	Yokogawa Analytical Systems IC7000
Injection volume	50 μl
Column	ICS-C25 (cation analysis)
Gird column	ICS-C2G
Eluate	5 mM tartaric acid/1 mM 2,6-pyridine dicarboxylic acid
Detector	Conductometric detector
Flow rate	1 ml/min
Column temperature	40°C

defined as the breakthrough volume. The breakthrough volume was measured to determine the optimum volume of the gas samples that can be captured by the adsorbent Tenax-TA. We evaluated the breakthrough volume of a series of two Tenax-TA tubes for each sample as reported previously[10] to determine the trapping capacity of Tenax-TA that we used in this study to trap the odor of excretion. Specified amounts (1 ppm, 1 μl) of the standard solution of fatty acid, pyridine, and pyrrole were added to Tenax-TA1 and then helium gas was passed through two Tenax-TA trapping tubes connected in series.

No breakthrough for butyric acid, *iso*-valeric acid, *n*-valeric acid, and pyridine was observed after 30 l of helium gas had passed through Tenax-TA. In the case of pyrrole, no breakthrough was observed until 10 l of helium gas had passed. Therefore, based on the detection limits, the exposure of Tenax-TA to at least 10 l of each target substance is thought to be appropriate.

Simultaneous Identification and Determination of the Concentrations of Malodorous Compounds from Human Feces Using TCT/GC/MS

After 80 l of air collected in the Tedlar bag was condensed to 10 l with Tenax TA, the sample was analyzed after adding 2 μl of 1 ppm deuterium-substituted benzene (C_6D_6) as an internal standard solution. To our knowledge, this is the first report on the identification and determination of malodorous substances of human feces immediately after using a flush toilet, and there is no other report on the malodorous substances in human diarrheal feces. Table 4 shows the results of our analysis of samples collected from individuals with normal excreta and those with diarrhea. In healthy subjects (samples 1–10), little difference was observed in the values obtained. The pyridine concentration was about 6 ppb for all samples. Acetic acid, propionic acid, butyric acid, *iso*-valeric acid, *n*-valeric acid, and pyrrole were detected at concentrations on the order of ppb, but indole and skatole, which are generally considered to be the primary malodorous components of feces, were not detected. The concentrations of pyridine, acetic acid, propionic acid, butyric acid, *iso*-valeric acid, and *n*-valeric acid were more than 10- to 100000-fold higher than normal levels when the subjects had diarrhea. Two reasons for this can be considered. There are many undecomposed proteins caused by abnormal fermentation and indigestion, and these undecomposed proteins form fatty acids and *N*-containing compounds. The foul smell is due to the feces floating in the water. These differences in the concentrations of malodorous components of feces depending on health conditions are extremely interesting.

Determination of Concentration of Sulfur-Containing Compounds

Hydrogen sulfide and methyl mercaptan were the only sulfur compounds detected (Table 5). The concentration of hydrogen sulfide was 5–26 ppb (n=7). Methyl mercaptan was detected at 2–15 ppb in only three of the seven subjects. The concentrations of both substances were lower than those in the malodorous gas from human waste reported previously,[10] probably because feces immediately after defecation is less decomposed by microorganisms than human sewage waste. However, as the thresholds of olfactory sensitivity[15,16] for methyl mercaptan and hydrogen sulfide are low, their odor intensities, which represent the human olfactory sensitivity to them, are high, and they are considered to contribute significantly to the odor of feces. Therefore elimination of methyl mercaptan and hydrogen sulfide is the most effective method of deodorizing the lavatory after defecation.

Although hydrogen sulfide was detected in all samples regardless of the health condition of the subjects, the presence of methyl mercaptan was found to be highly health dependent. Therefore me-

Table 4. Concentrations of Fatty Acids and N-containing Aromatic Compounds in Human Feces

Normal							
							(ppb)
Sample No.	Acetic acid	Propionic acid	Butyric acid	*iso*-Valeric acid	*n*-Valeric acid	Pyridine	Pyrrole
1	< 1	< 1	0.24	0.03	0.01	8	2
2	< 1	2	0.02	0.01	0.01	6	3
3	< 1	< 1	0.08	0.01	0.01	8	2
4	10	1	0.20	0.03	0.01	5	1
5	5	5	0.11	0.05	0.01	9	1
6	< 1	11	0.12	0.04	0.01	8	3
7	4	< 1	0.15	0.10	0.05	10	1
8	7	7	0.30	0.01	0.01	1	1
9	3	< 1	0.35	0.02	0.04	5	1
10	< 1	< 1	0.02	0.01	0.01	2	2
Ave	3	3	0.16	0.03	0.02	6	2
With diarrhea							
							(ppm)
Sample No.	Acetic acid	Propionic acid	Butyric acid	*iso*-Valeric acid	*n*-Valeric acid	Pyridine	Pyrrole
11	497	2.8	2.0	0.03	0.77	0.10	0.01
12	600	3.5	3.0	0.30	0.90	0.20	0.03
Ave	549	3.1	2.5	0.32	0.84	0.15	0.02

Table 5. Concentrations of Hydrogen Sulfide and Methyl Mercaptan in Human Feces

			(ppb)
Sample no.	Health condition	H_2S	MM
1	Normal	12.6	< 0.1
2	Normal	7.1	2.3
3	Normal	6.8	< 0.1
4	Normal	5.3	< 0.1
5	Normal	5.6	< 0.1
Ave		7.4	0.5
6	With diarrhea	25.5	14.6
7	With mild cold	6.0	2.7

thyl mercaptan might be produced only when the balance of the intestinal flora is disturbed. According to studies by Mitsuoka,[17,18] the odor of feces is related to disturbances in the balance between useful microorganisms such as *Bifidobacterium bifidum* and harmful microorganisms such as *Escherichia coli* in the intestinal flora, and to aging of the intestinal environment. Moreover, feces with a strong odor are now associated with future occurrences of colon cancer, arteriosclerosis, and liver disorders, and induces the progression of cell aging.

Differences in the components of odor were also observed based on the diet of the subjects. The consumption of leeks, onions, or asparagus is known to induce the production of methyl mercaptan.[19–21] In this study, methyl mercaptan was detected in a subject who ate quail eggs, and meals consumed the previous day were shown to affect fecal odor. This is considered to be due to the generation of sulfur compounds from proteins. According to a study by Hoshika *et al.*,[22] mercaptans, which are the primary causes of fecal odor, are closely associated with disease. Based on these results, the detection of sulfur compounds in the malodorous gas samples collected during defecation is considered to be important for evaluating not only the effects of an individual's diet, but also of general health. Therefore the quantitative monitoring of offensive fecal odor could be used as a self-health care method.[23]

Determination of Ammonia Concentration

Determination of ammonia concentration in the malodorous gas samples was performed 16 times for six subjects. Ammonia was detected in two gas samples after defecation at a mean concentration of 40 ppb, and in one of the seven samples of toilet room air at 120 ppb. It was also detected in one of the seven air samples collected directly from the toilet bowl at 40 ppb. These ammonia concentrations were not markedly different from the control value,

Table 6. Cocentrations of Malodorous Compounds in Human Feces

Type of compound	Compound	Concentration (ppb)
Sulfur-containing compounds[b]	Hydrogen sulfide	5–26
	Methyl mercaptan	2–15
	Methyl sulfide	nd[a]
	Dimethyl disulfide	nd[a]
Nitrogen-containing compounds[b]	Trimethylamine	0.01
	Ammonia	< 100
Aldehydes[b]	Formaldehyde	nd[a]
	Acetaldehyde	nd[a]
	Propylaldehyde	10
Fatty acids[c]	Acetic acid	3–10
	Propionic acid	2–11
	Butyric acid	< 0.4
	iso-Valeric acid	< 0.1
	n-Valeric acid	< 0.1
Others[b]	Pyridine	1–10
	Pyrrole	1–3

a) Not detected. *b*) Concentration of compounds under normal conditions and diarrhea. *c*) Concentration of fatty acids under normal conditions.

and the ammonia concentration after defecation was 100 ppb or less with respect to the detection limit using the indophenol method (1 mg/l) when 20 l of gas sample was aspirated.

When we analyzed the samples by ion chromatography to confirm the measured values, the ammonia concentration was determined to be 50–60 ppb in the samples of toilet room air (n=4) and 30–40 ppb for air samples collected directly from the toilet bowl (n=8).

The mean background concentration of ammonia in the atmosphere is estimated to be 6 ppb.[24] In urban areas, the atmospheric ammonia concentration is reported to be 10 ppb or less (with variations between sampling sites), and it has been shown to increase to approximately 200 ppb in buildings during the daytime.[24]

Therefore a small amount of ammonia was contained in the malodorous gas collected immediately after defecation, and the ammonia detected at trace levels may have been generated by nitrogen-containing compounds in the toilet and may have been present even before defecation. This ammonia was thus not generated by the intestinal flora. The ammonia odor in the lavatory is thought to be a product of the putrefaction process.

Determination of Concentration of Amines and Aldehydes

Trimethylamine was assayed by ion chromatography after the sample solution was adjusted to a neutral pH by adding 1 M NaOH. We found that the trimethylamine concentration was at the trace level, and when we used the gas chromatography method[14] to confirm the measured values, the trimethylamine concentration was determined to be 10 ppt (Table 6). Although high trimethylamine levels were detected in samples of raw sewage in our previous study,[10] the trimethylamine levels in the odor of excreta were found to be minimal in the present study. Therefore trimethylamine is considered to be generated by the decomposition of urine or feces.

Aldehyde analysis was performed for only one sample. Only one peak was obtained, and it corresponded to propylaldehyde based on the retention time of the standard. The aldehyde concentration was about 10 ppb (Table 6). The contribution of this component to the odor of feces is considered to be similar to that of fatty acids.

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