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Deodorization of Pig Feces by Actinomycetes

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Pig feces, a malodorous substance causing environmental pollution, were completely deodorized within 2 days by *Streptomyces*. The optimum conditions for deodorization were as follows: pH, 8.6 to 10; temperature, 35 to 40°C; moisture content, 42 to 63%; and minimum amount of inoculum, 2 g of seed culture per 10 g of fresh feces. Many kinds of microorganisms were isolated from the deodorized feces, of which only actinomycetes were found to have the ability to deodorize. Two strains with strong deodorizing activity were identified as *Streptomyces griseus* and *Streptomyces antibioticus*. The low-molecular-weight fatty acids, which are the specific malodorous agents of pig feces, scarcely could be found in feces deodorized by the isolated strains. Chemical analysis showed that the deodorized feces are useful as manure.

Malodor generated by domestic animal houses is one of many causes of environmental pollution. In recent years, due to the highly developed techniques of animal husbandry to raise a large number of domestic animals in one place and the decrease in use of excrement as a manure because of popularization of chemical fertilizer, large amounts of the excrement of domestic animals have accumulated and have polluted the environment.

Today, excrement is treated in a treatment plant with activated sludge (3) or dried in the sun or with artificially heated wind to make a dry manure (8). Some types of yeasts are used to treat chicken feces (7). However, these treatment methods require a large-scale plant, high costs, or a long time.

One of the most difficult problems in the treatment of pig feces is that they have both malodor and high moisture content (usually 73 to 85%).

One attempt has been made to deodorize the pig feces with microorganisms. Fortunately, a farmer was found in Fukuyama City, Hiroshima, Japan, who has raised 200 to 300 pigs and has treated their feces with a microbial culture to deodorize and dry them and then used the treated feces (odorless and dried) as a seed culture to treat fresh feces and as manure for crop fields for more than 10 years. However, it has never been determined what kinds of microorganisms deodorize, what conditions are most suitable for deodorization, or what properties the microorganisms have.

Some reports have been published on the treatment of pig feces (3, 7), but all of them concern how to make barnyard manure. We are not aware of any studies on the deodorization of pig feces by microorganisms. This paper is concerned with the isolation and identification of the microorganisms that deodorize the pig feces, the most suitable conditions for deodorization, and the physiological properties of the microorganisms.

MATERIALS AND METHODS

Pig feces and seed culture. Feces were collected, within 2 days of excretion, from pigs fed with concentrate. The seed culture used for the inoculum in this experiment was the deodorized feces with some kind of microorganisms (odorless, with a slightly white surface), which have been made and used by the farmer to treat fresh pig feces experimentally for more than 10 years. For inoculum, the deodorized feces used as seed culture were cracked and powdered in a mortar with a pestle. This sample was also used for the isolation of microorganisms which were used to deodorize the fresh feces in this experiment.

Determination of pH. About 10 g of a sample was suspended in 100 ml of distilled water. The pH was determined with a pH meter (Hitachi Co., model M-5) with stirring on a magnetic stirrer.

Determination of malodor. (i) Qualitative determination. The malodor was detected qualitatively by human volunteers. Malodor strength (MS) was defined on a 0 to 5 scale, where the MS of normal raw and fresh pig feces was MS 3. Stronger odors were given higher values, and the weaker odors were given lower values, with MS 0 being below detection of the characteristic odor of fresh feces (sometimes different odors such as microbial odors were given by samples designated MS 0). The reported scores were obtained by treatment of the individual scores by the method of least squares, after eliminating the extremes.

(ii) Quantitative determination. Determination

of some typical ordinary malodors, e.g., ammonia, hydrosulfite, mercaptoethanol, were determined quantitatively with gas-detecting tubes containing gas-sensitive material for each malodorous gas (Kitagawa Co., Tokyo, Japan). A 100-g amount of the sample was kept in a 1-liter Erlenmeyer flask with a rubber stopper for 1 h at 40°C, and then 100 ml of the air in the flask was introduced into the gas-detecting tube (diameter, 2.5 mm) with a pump connected to the tube. The length of the color-changed part in the tube was measured, and the malodor content was determined by comparison with the standard chart.

(iii) Gas chromatography of the malodors of pig feces. A 10-g amount of the feces sample was acidified to pH 1 with 2 ml of 1 N HCl and extracted with 2.5 ml of ether. After storing at −20°C for 4 h, then 1 ml of the ether layer containing the low-molecular-weight fatty acids, which are the main components of the malodors of the fresh pig feces (2), was taken and treated with diazomethane (4). The methylated sample was subjected to gas chromatography.

Isolation of microorganisms. The sample taken from several parts of the deodorized feces was mixed well and ground to powder in a mortar and pestle. Then, 1 g of the powdered sample was suspended in 10 ml of sterile saline solution (0.9% NaCl) in a test tube. After coarse materials settled by standing for about 10 min, the suspension was taken for isolation of microorganisms. All microorganisms were isolated at 30°C. The media used for isolation of microorganisms were as follows. Nutrient agar (Difco Laboratories, pH 6.8) and malt extract agar (Difco, pH 5.5) with 100 μg of streptomycin per ml were used for isolations of bacteria and of fungi and yeasts, respectively. For isolation of actinomycetes, 10% pig feces extract agar medium (PFE medium) was employed. This medium was prepared as follows. One part of the fresh feces was suspended in 10 parts of distilled water, the suspension was filtered through absorbent cotton, and the filtrate (pH was about 7.3) was solidified with agar powder (1.5%). All media were sterilized at 121°C for 15 min.

Enumeration of microorganisms. The sample treatment and the media used for the counting of microorganisms were as described above; 10-fold dilutions were made.

Chemical analysis of the feces. Moisture content was determined by drying a portion of the sample at 115°C to constant weight. Crude protein content and inorganic ammonia were determined by semimicro-Kjeldahl procedure (6). The crude lipid content was determined with Soxhlet extractor by the standard method (6). The crude fiber content was determined as follows. After hydrolysis of a portion of the sample in 1 N HCl in a reflux distiller, the hydrolysate was filtered through a sintered glass filter. The residue was washed with distilled water until chloride was undetected. Then, the residue was dried at 115°C to constant weight. Phosphate content was determined by the method of Fiske and Subbarow as modified by Nakamura (5) after the sample was mineralized to inorganic phosphate with heated sulfuric acid. Potassium content was determined by flame spectrochemically (6).

RESULTS

Optimum conditions for deodorization. **(i) Optimum pH.** A 10-g amount of fresh pig feces was mixed with 1 g of the powdered seed culture, and 1 ml of various alkaline or acidic solutions were added to adjust the pH of the mixture to 3.5, 4.3, 5.3, 6.4, 7.3 (the fresh feces themselves), 7.5, 8.6, 10.0, 11.0, and 11.5. Each sample was poured in a petri dish and kept at 30°C. The pH-adjusted feces, without addition of seed culture, were kept at the same temperature for the control experiment. The malodor was detected at 1-day intervals for 7 days. Merely by adjustment of pH of the feces to the acidic side, the malodor became stronger, and the black color changed to yellowish; on the other hand, when the pH was adjusted to alkaline side, the malodor was just slightly reduced. This suggests that the malodorous agents of pig feces are acidic substances. With the seed culture, the malodor of the feces with pH values between 8.6 and 10.0 disappeared after incubation at 30°C for 3 days. The malodor of uninoculated feces did not disappear at any pH value tested even after 7 days at 30°C.

(ii) Optimum temperature. Each 10-g portion of the fresh pig feces was inoculated with 1 g of the powdered seed culture, poured in petri dishes, and kept at 10, 20, 30, 35, and 50°C for 7 days. For the control experiment, the feces without addition of seed culture were kept at the same temperatures as described above in petri dishes for 7 days. The malodor of pig feces with seed culture completely disappeared between 35 and 40°C for 5 days. However, the malodor of the uninoculated feces did not disappear at any temperature shown above, even after 7 days.

(iii) Effect of the amount of the inoculum. To 10 g of the fresh pig feces, 0.5, 1, 2, 3, and 5 g of the powdered seed culture were added, respectively. Then, they were poured in petri dishes and kept at 30°C. The malodor of the feces with more than 2 g of the inoculum to 10 g of the fresh pig feces disappeared after 2 days. The malodor of the uninoculated pig feces did not disappear even after 7 days.

(iv) Effect of moisture content. Fresh feces with a moisture content of 75 to 85% were dried at 60°C to a 10% moisture content (taking about 2 days). Even after drying, the MS did not change; MS was about 3. Distilled water was added to a moisture content of 10, 25, 42, 63, 71, 75, or 77%. After addition of the seed culture (10%), the samples were incubated in petri dishes at 30°C. The malodor of the feces with a moisture content between 42 and 63% disappeared completely after 3 days. The malodor of the

uninoculated pig feces with various moisture contents did not disappear.

Generally, the whole or parts of the surface of the deodorized feces became slightly white, and the feces had a microbial odor. The microbial growth during deodorization was investigated.

Microbial growth during deodorization of pig feces. The fresh feces were deodorized under the folowing conditions. A 100-g amount of fresh pig feces (pH 7.3) was inoculated with 20 g of powdered seed culture and kept at 40°C in a petri dish (diameter, 18 cm). At 1-day intervals, 10 g of the sample was withdrawn from the petri dish, and microbial growth was investigated. The results are shown in Fig. 1. The malodor disappeared completely after 2 days. Numbers of bacteria, fungi, and yeasts decreased. However, the number of actinomycetes increased. The surface of the deodorized feces became slightly white, and the odor changed to that of soil.

Isolation and characteriation of microorganisms. With nutrient agar medium, malt agar medium containing streptomycin, and PFE agar medium, 20 strains of bacteria, 16 strains of fungi, 20 strains of yeasts, and 49 strains of actinomycetes were isolated at 30°C from the deodorized feces and the seed culture. Also, 20 strains of thermophilic bacteria were isolated at 55°C on nutrient agar medium. Each strain was used to inoculate 1 g of autoclaved feces (121°C, 15 min) in a test tube with cotton plug and was incubated at 30°C, except that the thermophilic bacteria were incubated at 55°C. All strains of actinomycetes deodorized the feces. The other microorganisms isolated had no deodorizing activity. All of the 49 strains of actinomycetes deodorized the pig feces within 2 days and had grayish to white aerial mycelium with straight or wavy chains of spores. None showed fragmentation of submerged mycelium. Most showed optimum growth at 40 to 45°C (range, 30 to 50°C) and at a pH of 9. Nitrate reductase activity was variable. They were morphologically and physiologically identified to be *Streptomyces* according to *Bergey's Manual of Determinative Bacteriology*, 8th ed. (1). Strains 10b and 104, which were the most active in deodorizing feces, were similar to *Streptomyces griseus* and *Streptomyces antibioticus*, respectively, based on their morphological, physiological, and biochemical characteristics.

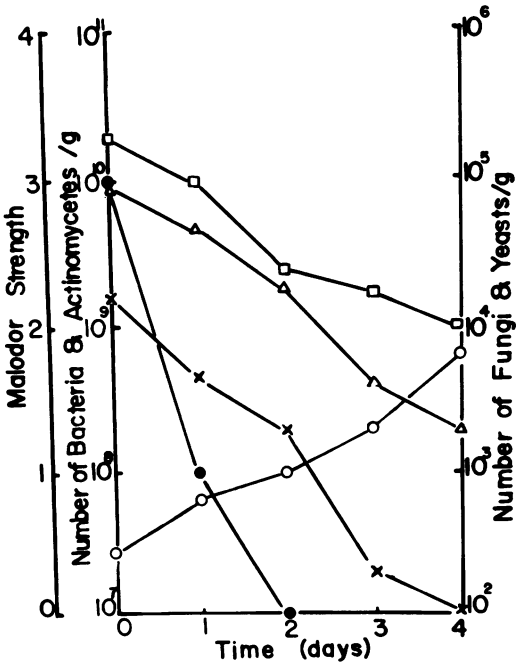

FIG. 1. *Microbial growth (cells per gram) during deodorization of pig feces. See the text for the conditions for deodorization. Symbols:* □, *bacteria;* △, *yeasts;* ×, *fungi;* ○, *actinomycetes;* ●, *MS.*

Gas chromatography of fecal extract. Fresh feces (20 g) were inoculated with strain 104 and deodorized after incubation for 2 days at 40°C. The deodorized feces and the fresh feces were subjected to gas chromatography. The results are shown in Fig. 2. The low-molecular-weight fatty acids, which are the main components of the malodor of pig feces (2), were detected in the fresh feces, but were barely detectable in the treated (deodorized) feces.

Analysis of malodors with gas-detecting tubes. The deodorized pig feces (100 g) with strain 104 after incubation for 3 days at 40°C in a petri dish (diameter, 18 cm) and the fresh feces (100 g) were used for quantitative analysis of some ordinary malodorous agents by using gas-detecting tubes. The results are summarized in Table 1. The amount of hydrosulfite decreased 725 to 18 ppm. Also, mercaptoethanol decreased from 4 to 1 ppm. On the other hand, ammonia gas increased 2 to 30 ppm. Ammonia might be generated because of the rise in pH of the treated feces or due to death of the microorganisms and deamination. Methylmercaptan was not detected in deodorized feces. Formaldehyde and SO_2 were not detected in either fresh or deodorized feces.

Chemical analysis of the fresh and the deodorized feces. A 100-g amount of fresh pig feces (pH 7.3) was inoculated with 20 g of powdered seed culture and incubated in a petri dish (diameter, 18 cm) without the lid at 40°C for 2 days. Thus, deodorized pig feces and the fresh feces were used for the analytical sample. The

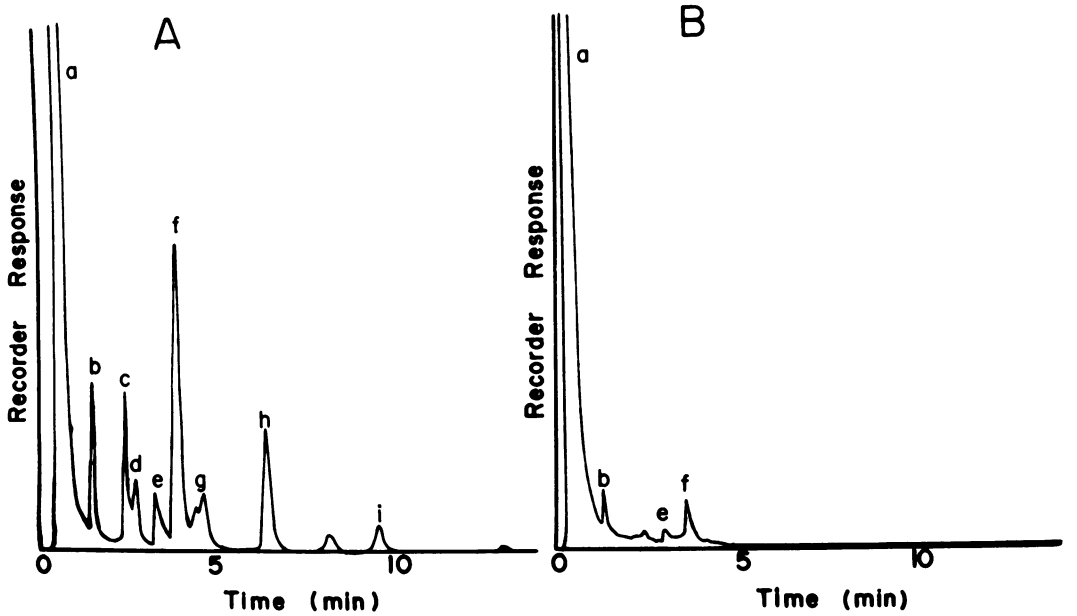

FIG. 2. *Gas chromatography of the fresh and deodorized feces. (A) fresh feces; (B) deodorized feces after incubation with strain 104 for 2 days at 40°C. Symbols: a, solvent (ether); b, acetic acid; c, propionic acid; d, isobutyric acid; e, methanol; f, ethanol and n-butyric acid; g, isovaleric acid; h, n-valeric acid; i, caproic acid. Chromatographic conditions: sample size (methylated), 2 μl; column, polyethylene glycol 1500, 3 by 1,500 mm, 35 to 120°C (4°C/min); detector, flame ionization detector; carrier gas, N_2 (30 ml/min); fuel gas, H_2 (30 ml/min); air, 1 liter/min.*

TABLE 1. *Comparison of the concentration of malodor component in untreated and deodorized feces*

Malodor component	Concn in fresh feces (ppm)	Concn in deodorized feces (ppm)
NH_3	2	30
H_2S	725	18
CH_3SH	15	ND[a]
C_2H_5SH	4	1
CH_3CHO	ND	ND
SO_2	ND	ND

[a] ND, Not detectable.

pH of fresh feces rose from 7.3 to 9.0, and the moisture content decreased from 85 to 25% during deodorization. The feces might be dehydrated because the lid of the petri dish was not used during incubation. The contents of crude protein, fat, and fiber were decreased by the deodorization. However, the nitrogen-free extract increased, probably due to the decomposition of crude fiber by the microorganisms. Cellulolytic microorganisms might be present in the seed culture because the content of crude fiber was decreased. The total nitrogen decreased, whereas total K_2O and P_2O_5 increased. These contents are lower than those of chicken feces, but the content of the nitrogen was same as chicken. Dried chicken feces obtained commercially were also analyzed for comparison. The results are shown in Table 2.

DISCUSSION

By using the petri dish assays, the optimum conditions for several parameters for deodorization of the pig feces were determined. These were (i) pH values of 8.6 to 10.0, (ii) temperature between 35 and 40°C, (iii) moisture content of 40 to 63%, and (iv) minimum inoculum size of 20% (2 g of seed culture per 10 g of fresh feces). The pH and temperature ranges are coincident with those obtained with the pure culture of the isolated *Streptomyces* sp. The pH of the fresh feces is about 7.3, but the older the feces become, the lower the pH becomes, and the malodor becomes much stronger, presumably due to increased protonation of various volatile organic compounds. Therefore, it is necessary to treat the fresh feces as soon as possible after excretion. Because the moisture content is suitable between 42 to 63%, the urine and the feces should be separated in animal houses. The isolated microorganisms were inoculated on the sterilized feces, and only actinomycetes group was found to have deodorizing activity. Low-molecular-weight fatty acids, which cause the specific mal-

TABLE 2. *Chemical analysis of fresh and deodorized feces*

Feces	pH	Moisture content (%)[a]	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Crude ash (%)	Nitrogen-free extract (%)	Total nitrogen (%)	Total P_2O_5 (%)	Total K_2O (%)	Inorganic NH_3 (mg/g)[b]
Fresh	7.3	73	22.1	12.1	39.5	19.3	7.0	3.5	1.2	0.6	3.8
Deodorized	9.0	25	15.7	1.2	11.0	33.3	38.7	2.5	2.7	1.6	3.3
Chicken[c]	9.5	23	15.7	1.1	17.7	33.9	29.9	2.5	4.4	5.4	

[a] Percentages are per dry matter.
[b] In dry matter.
[c] Chicken feces dried with artificially heated wind and used as a manure were commercially obtained.

odors of the feces, could not be detected in the feces treated with *S. antibioticus* (strain 104), although they are detected in the fresh feces, as shown in Fig. 2A and B. These fatty acids are probably metabolized by the organisms, or enzymes secreted by the organisms, any of which could result in deodorization. The ordinary malodorous agents, such as hydrosulfite and ethylmercaptan, were found to be decreased in the deodorized feces with strain 104 by using the gas-detecting tubes, although ammonia gas was generated. The ammonia gas might be generated because of the rise in pH or microbial deamination. The same results were obtained with feces deodorized with the seed culture (a mixture of many kinds of microorganisms). The strains of *Streptomyces* sp. in the seed culture worked as deodorizing microorganisms.

Chemical analysis of the treated (deodorized) feces showed that the nitrogen content decreased. This might be due to the consumption of nitrogenous compounds by the microorganisms, followed by the generation of ammonia. The crude fiber decreased, and the nitrogen-free substances increased; by the decomposition of the crude fiber, the nitrogen-free substances might be increased consequently. Because the amount of nitrogen in the deodorized feces was almost the same as that of the commercial chicken feces, it could be used as a manure, although the amounts of P_2O_5 and K_2O were less than those of the chicken feces.

The mechanisms of the deodorization of pig feces need to be investigated, as well as a feasibility study of a large-scale process. Also, it should be determined whether the actinimycetes isolated are capable of deodorizing other kinds of feces.

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