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Utilization of Deodorized Poultry Feces with *Tolura* sp. CH-30

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Treatment of poultry feces with *Tolura* sp. CH-30 produced a material that was significantly deodorized and showed a promotive effect on plant growth. *Tolura* sp. CH-30 grew on the poultry feces, deodorizing the feces by assimilation of volatile fatty acids, which are the source of the offensive odor, as a carbon source. Significant degradation of uric acid also occurred. In the treatment of feces with *Tolura* sp. CH-30, it was possible to deodorize feces in a short time, but reduction in the amount of urate-N was not enough. Urate-N inhibited plant growth due to an excessive nitrogen content produced as a result of rapid decomposition. Therefore, we propose a recycle-treatment plan using poultry feces treated with *Tolura* sp. CH-30. After the recycle-treatment, the amount of urate-N contained in the recycle-treated poultry feces was small and the recycle-treated poultry feces showed a promotive effect on plant growth when it was added at a nitrogen content of 1.6 g/600 g soil/pot.

With the development of the livestock industry, large amounts of domestic animal excrement have accumulated. In agriculture, the use of excrement as barnyard manure has been decreasing because of popularization of chemical fertilizers. As a result, excrement has created environmental pollution such as the contamination of water resources and production of obnoxious odors. To solve these problems, many researchers are studying the treatment of livestock excrement (3, 4, 12-14), especially of pig feces (7, 10). Hayashida *et al.* (7-9) reported that swine feces was deodorized in a practical, semi-continuous treatment by using a mixed culture of mesophilic and thermophilic actinomycetes. The treated swine feces was then used as fertilizer. We tried to produce a good fertilizer from poultry feces, but it was difficult for two reasons. First, microorganisms usually did not grow well on fresh poultry feces without sterilization or the use of additives and, second, plant growth was inhibited by the excessive nitrogen content produced as a result of rapid decomposition of nitrogen compounds in poultry feces, such as uric acid, containing 70 to 80% total nitrogen (6).

This study was concerned with the deodorization of poultry feces with *Tolura* sp. CH-30 and the production of a usable fertilizer using treated poultry feces.

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Key words: Deodorized poultry feces, recycle-treatment

MATERIALS AND METHODS

Screening of Microorganisms Available for Composting of Poultry Feces

For the screening of urate-degrading microorganism strains, plates containing poultry feces-extract agar medium were inoculated with diluted suspensions of various samples of composed livestock feces. One thousand grams of poultry feces was added to 1000 m*l* of tap water, left to stand for 10 minute at room temperature, filtered through a sheet of gauze, supplemented with 1.5% agar, and then adjusted to pH 8.5 with 2 N Na_2CO_3 before sterilization at 121℃ for 30 minute. After inoculation, the samples were incubated at 30℃ for 5 days. Isolated strains were tested for growth potential on unsterilized fresh poultry feces containing 23% (w/w) of air-dried poultry feces and 1.5% (w/w) of $Ca(OH)_2$. The moisture content was 60% (wt/wt). The strains were secondly isolated for uricase activity on a uric acid agar medium (5). Determination of pH and temperature for growth of the newly isolated mesophilic fungal strain CH-30 were carried out using poultry feces-extract agar medium. The morphology of strain CH-30 was observed with Nikon microscope.

Gas Chromatography of the Odors

Volatile fatty acids, which are the main compounds of poultry feces odor, were analyzed quantitatively by gas chromatography. Fresh poultry feces (50 g wet matter) and poultry feces treated with strain CH-30 for 24 and 72 hours (50 g wet matter) were mixed with 50 ml of distilled water. These samples were acidified to pH 2 with 2 N H_2SO_4. Volatile fatty acids from each sample were collected in a 50 ml volumetric flask by distillation, and their amounts were immediately estimated by gas chromatography with a glass column packed with KOCL-3000T 1% Greensorb F 40/60, Yanaco. Nitrogen was used as a carrier gas at a rate of 30 ml/min. The column oven temperature was kept at 190℃ and the injection port was kept at 210℃.

Composting Process of Poultry Feces

The selected strain was inoculated onto a slant of poultry feces-extract agar medium and then incubated at 30℃ for 5 days. The spores were inoculated onto a wheat bran medium containing 25 g of wheat bran, 10 g of fresh poultry feces, 40 ml of tap water and 1 g of $Ca(OH)_2$. The moisture content was 60% in a 500 ml Erlenmeyer flask. Inoculation was followed by incubation at 30℃ for 2 weeks for the seed culture. The seed (1×10^{10} viable count/g) was inoculated at 5% into a mixture of 5 kg of fresh poultry feces, 1 kg of air-dried poultry feces, and 90 g of $Ca(OH)_2$. The moisture content was 60% without sterilization and any additives were in a shallow plastic pan ($0.9\times1.5\times0.2$ m). Inoculation was followed by incubation at 30℃ for 20 days. In order to reduce the uric acid content and to treat poultry feces continuously, a recycle-treatment was carried out. With the process of recycle-treatment, the poultry feces treated with *Tolura* sp. CH-30 was substituted for both seed culture and air-dried poultry feces. The treatment process was the same for both.

Analytical Methods

The pH was measured with pH meter (Model SA 520 Orion). Ten grams of a sample was suspended in 100 ml of distilled water, left to stand for 10 minutes at room temperature and then the pH was measured. The moisture content was measured with a moisture meter (Kett Co. Japan). Viable counts of *Tolura* sp. CH-30 and coliform bacteria were determined in the poultry feces extract and the desoxycholate agar medium by the plate dilution method. The total amount of carbon and nitrogen were determined by means of dry combustion using a Yanaco CN corder (MT 500). Urate-N and protein-N were measured by piperazine method (5) and amino acid analysis using HPLC (Hitachi 638-30), respectively. Quantitative analysis of P_2O_5, K_2O and inorganic nitrogen, such as NH_4-N and NO_3-N was done according to the soil and plant analytical methods (2).

Pot Experiments

To assess the effectiveness of treated feces as fertilizer, pot experiments were carried out in triplicate. The soil was passed through a 16 mesh sieve. The pH of the soil was adjusted to pH 6.2 with $CaCO_3$ and the moisture content was maintained at 60% of the water holding capacity. Treated poultry feces, recycle-treated poultry feces, air-dried poultry feces, rapeseed meal and ammonium sulfate were used as fertilizers. The fertilizer samples were air-dried and crushed into pieces of about 1 mm in diameter before application. The nitrogen content was varied in steps from 0, 0.1, 0.2, 0.4, 0.8 and 1.6 g N/600g soil/pot. Six hundred grams of soil was mixed with a sample and then poured into a 1 liter plastic pot. Twenty five seeds of chinese mustard (*Brassica rapa* var. *peruiridis*) were sprinkled uniformly over the surface of each pot. Water was added to the pots every day in order to maintain 60% of water holding capacity. Random placement rotation of the pots was also carried out every day to minimize location effects. All pots were cultivated at 25℃ in a greenhouse. Fresh weight was determined after a 30 day-cultivation.

RESULTS AND DISCUSSION

Selection of Fungus Useful for Composting of Poultry Feces

Selected strain CH-30 showed predominate growth on unsterilized fresh poultry feces without any additives. Uricase activity was shown by the formation of clear lysis zones around colonies of strain CH-30 on a uric acid medium within 72 hours at 30℃, as shown in Fig. 1. The predominate growth of selected strain CH-30 was observed at temperature range 15 to 30℃ and pH value 4.5 to 9.5 on poultry feces-extract agar medium (Fig. 2). From the microscopic observation in Fig. 3 hyphae of the fungus was septate, richly branched, with short hyalin lateral branchlet, on which the conidial chains occur. Conidia bound in chains which break apart in single cells or in short pieces, 6~8 μ in diameter, globose, rough to warty. According to *the fungi* (1) the fungus was morphologically identifies as *Tolura* sp. CH-30.

Use of Fatty Acids by *Tolura* sp. CH-30

Treated poultry feces and fresh poultry feces were subjected to gas chromatography. The results are shown in Table 1. Volatile fatty acids odor, which are the main compounds poultry feces, were diminished owing to their use by *Tolura* sp. CH-30. Among the volatile fatty acids acetic acid, contained in large amounts in fresh poultry feces, was decreased 90% after 3 days treatment with *Tolura* sp. CH-30.

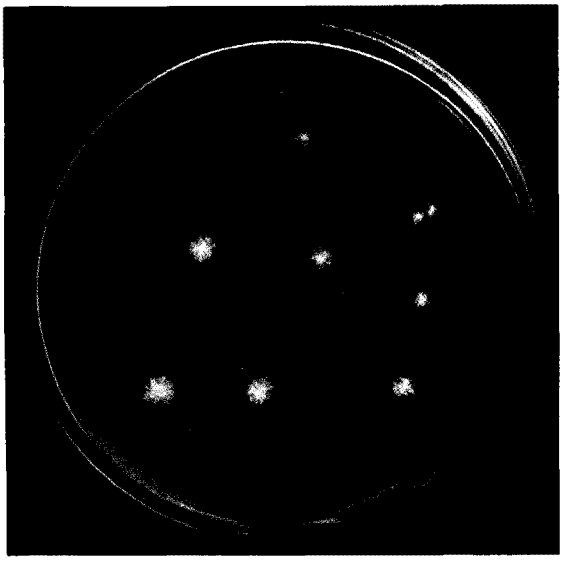

Fig. 1. Uricase activity of strain CH-30 on a uric acid medium.
Medium: uric acid 0.5%, yeast extract 0.1%, CH_3COOK 0.5%, agar 1.5%

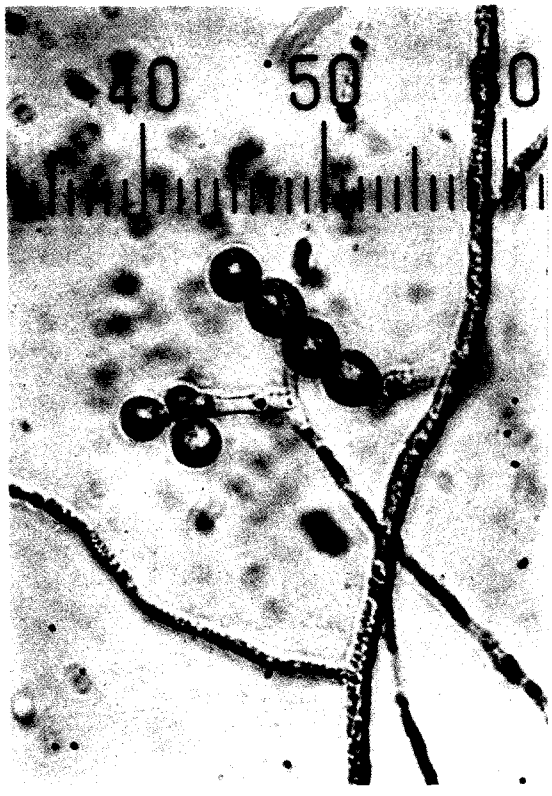

Fig. 2. The micrograph of spore chains of strain CH-30.
One division of the scale is 2.7 μm.

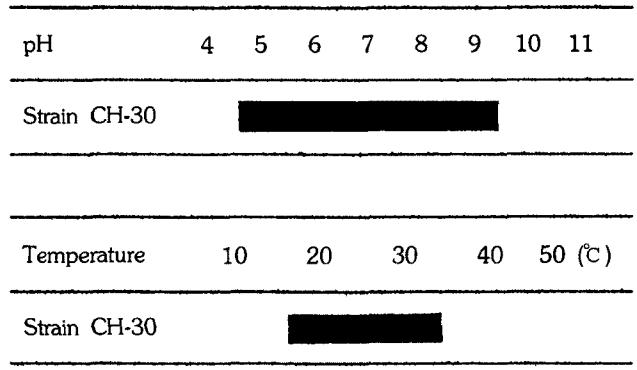

Fig. 3. Effect of pH and temperature on growth of strain CH-30.

Table 1. Changes of volatile fatty acids during treatment.

	V.F.A. composition (mg/100g dry matter)						
	C_2	C_3	C_4	C_5	iC_5	C_6	iC_6
Fresh poultry feces	88.1	34.8	22.8	5.4	7.2	2.4	3.4
Treated poultry feces							
24 hr	11.0	1.4	1.2	0.8	1.6	0.6	1.0
72 hr	5.6	0.4	0.4	0.5	0.3	0.3	0.6

C_2: Acetic acid, C_3: Propionic acid, C_4: Butyric acid, C_5: Valeric acid, iC_5: Isovaleric acid, C_6: Caproic acid, iC_6: Isocaproic acid

Composting Process of Poultry Feces with *Tolura* sp. CH-30

The changes in microbial population, moisture and chemical composition during poultry feces treatment are shown in Fig. 4. After 24 hours cultivation poultry feces were covered with the white hyphae of *Tolura* sp. CH-30, and were deodorized. After 96 hours of cultivation the feces were covered with the dark yellow conidia of *Tolura* sp. CH-30. Viable counts of *Tolura* sp. CH-30 increased from 6×10^6/g to 2×10^{10}/g wet matter within 12 days. After 20 days the final viable count of *Tolura* sp. CH-30 was 7×10^{10}/g wet matter. On the other hand, the viable counts of coliform bacteria decreased to 10^0/g wet matter after 4 days. After 20 days the urate-N and total-N amounts decreased from 26 mg to 9 mg and from 4.7% to 1.8%, respectively, but the C/N-ratio increased from 6 to 13 per 1 g dry matter. Finally, a treated poultry feces product containing 1.5% N, 3.3% P_2O_5 and 3.1% K_2O was obtained.

In order to reduce the uric acid content of the poultry feces and to continuously produce treated matter a recycle-treatment was carried out. In Fig. 5 the changes in microbial population, pH and chemical composition of

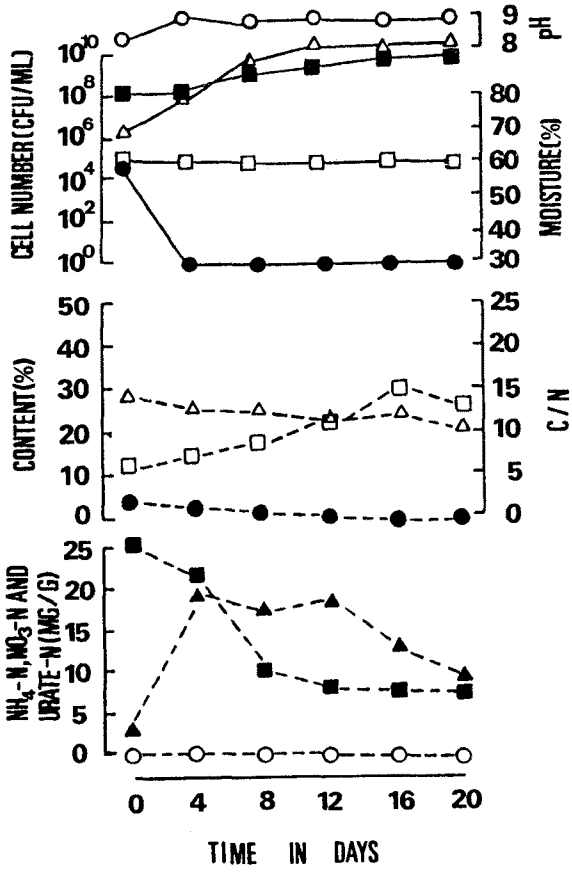

Fig. 4. Changes in microbial population, moisture and chemical composition during treatment.
Symbols: *Tolura* sp. CH-30; (△), coliform bacteria; (●), pH; (○), moisture; (□), total organic; (−△−), C/N; (−□−), total-N; (−●−), bacteria; (■), urate-N; (−■−), NH_4-N; (−▲−) and NO_3-N; (−○−)

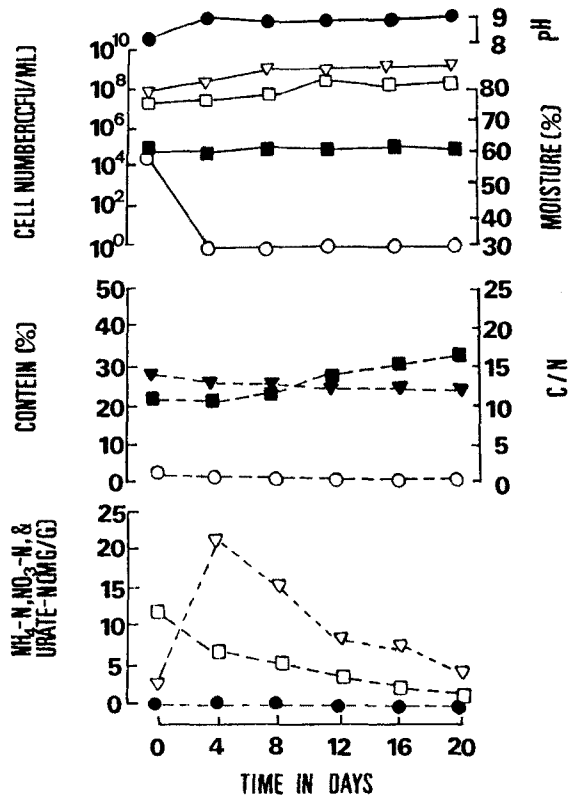

Fig. 5. Changes in microbial population, moisture and chemical composition during recycle-treatment.
Symbols: *Tolura* sp. CH-30; (▽), coliform bacteria; (○), pH; (●), bacteria; (□), moisture; (■), total organic; (−▼−), total-N; (−○−), C/N; (−■−), total-N; (−□−), NH_4-N; (−▽−) and NO_3-N; (−●−)

Table 2. Changes in chemical composition during treatment.

		Total organic-C	Total-N	Urate-N	Protein-N	C/N
Treatment	0 time	297	47	26	5	6
	20 days	231	18	9	5	13
Recycle-treatment	0 time	289	24	12	8	12
	20 days	264	15	2	5	18

(mg/g dry matter)

poultry feces during recycle-treatment are shown. The amount of urate-N per 1g dry matter decreased from 12 mg to 2 mg during treatment. After 20 days total organic carbon was slightly reduced but total nitrogen decreased significantly as a large amount of ammonia gas was evolved. Finally, recycle-treated poultry feces containing 1.5% N, 3.2% P_2O_5, 3.1% K_2O and a C/N ratio of 18 was obtained. The changes in compostion during treatment are shown in Table 2.

After treatment, the total organic carbon content and the total nitrogen content decreased. Many studies (5, 11, 15) have shown that the application of immature composts to the soil will cause sometimes severe damages to plant growth owing to nitrogen stravation and creation of anaerobical environment. It is thus necessary to determine whether or not the compost has been sufficiently matared. In general it was disclosed that with the progress of the composting process the ratio of organic car-

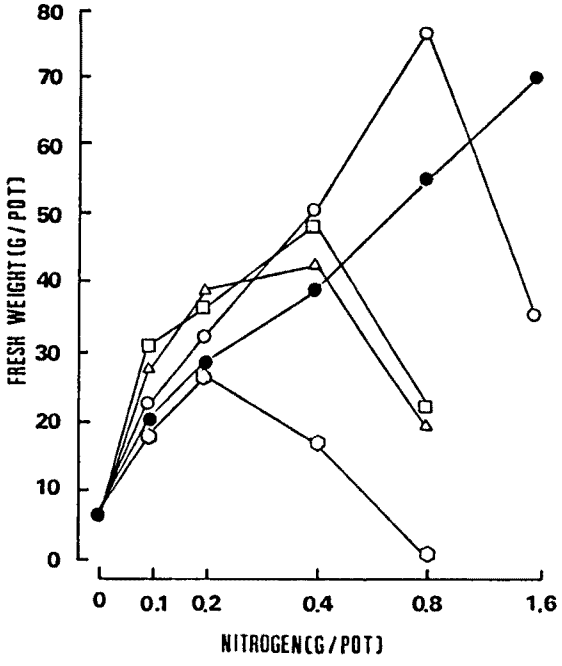

Fig. 6. Fres-weight yields of *Brassica rapa* var. *perviridis* fertilized by recycle-treated poultry feces.
(●), treated poultry feces; (○), air-dried poultry feces; (□), rapeseed meal; (△) and ammonium sulfate; (□).

bon content to organic nitrogen content approached to ranging from 13 to 18. In our experiment similiar results were also observed.

Pot Experiments

Fresh weights of chinese mustard (*Brassica rapa* var. *perviridis*) after a 30 day cultivation with various nitrogen contents are shown in Fig. 6. The highest yield obtained in the series of plants fertilized with ammonium sulfate was with the plant cultivated at a nitrogen content of 0.2 g N/pot. Plant growth at a high nitrogen content was suppressed. In the series with rapeseed meal and air-dried poultry feces, both yields were increased step by step up to a nitrogen content of 0.4 g N/pot, but yields were suppressed at a nitrogen content of 0.8 g N/pot. On the contrary, the yields of plants fertilized with both treated poultry feces and recycle-treated poultry feces increased step by step up to nitrogen contents of 0.8 g N/pot. The recycle-treated poultry feces had especially little effect with a small amount of fertilizer, and was only effective at a nitrogen content 1.6 g N/pot. The reason for these results was presumed to be that treated poultry feces contain most of their nitrogen as microbial mycelia, and these mycelia are gradually decomposed and continually supply the plant with proper amount of nitrogen. It is suggested that large amounts of treated feces could as an excellent fungus biofertilizer and soil-improving agen.

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