Kor. J. Appl. Microbiol. Biotech.
Vol. 18, No. 5, 530-534 (1990)

Treatment of *Thermoactinomyces* sp. to Application of Poultry Feces

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계분을 이용하기 위한 *Thermoactinomyces* sp. 균처리

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A strain of actinomycetes, *Thermoactinomyces* sp. CH-53, was isolated from manure and composted livestock feces. Actinomycetes-feed additive was prepared with the solid wheat bran medium of *Thermoactinomyces* sp. CH-53 that grew vigorously on unsterilized poultry feces at 50°C, pH 6.5-9.5 and moisture content of 55-65% and added at a rate of 1% (wt/wt) to the commercially assorted feed to be fed poultry. The excreted feces contained 10^7-10^8 *Thermoactinomyces* sp. CH-53 cells per gram. Poultry feces malodour was got rid of during treatment. The effect on plant growth was evaluated on the basis of the amount of nitrogen as fertilizer under a loading of 0.2g N/600g soil/pot. All samples were showed a promotion effect for plant growth. The treated poultry feces added from 0.1g to 0.4g total nitrogen per 600g soil in a pot increased the growth of *Brassica rapa* var. *perviridis*.

The livestock wastes in Korea are accumulated at the rate of 4 million tons (dry matter) annually. Such large production brings about severe pollution problems arising from the fact that large amounts of the wastes are accumulated on diminishing areas instead of spreading over agricultural land.

Development of units with an insufficient acreage for manure application enhances the risk of water and soil pollution. Rearing of animals on slatted floors, chosen for economic (straw price, less labour per animal) and social reasons (lack of interest in handling wastes) leads to production of a liquid slurry unable to stablize and constituting a considerable source of malodours (1-4).

Traditionally in Korea, the livestock wastes were treated under anaerobic and thermophilic conditions, and it took several months to be treated. The resulting manures produce obnoxious odors such as volatile fatty acid (5, 6), amine (7, 8), ammonia (9, 10), phenol (11, 12) and dimethyl disulfide (13, 14).

Today, the livestock wastes are treated in a treatment plant with activated sludge (15), dried in the sun, or with artificially heated wind to make a dry manure (16). Tanaka *et al.* (17) reported the method for livestock wastes treatment with fungi and yeast. Some reports have been published on the treatment of poultry feces using solid state fermentation equipment (18, 19).

This paper describes the research in which spores of the isolated strain, *Thermoactinomyces* sp. CH-53, were added to a commercial feed and the feed containing the spores was fed directly to poultry in order to determine the practical treatment of poultry feces and the effectiveness of the treated feces as fertilizer.

Materials and Methods

Screening of actinomycetes avialable for composting of poultry feces

Key words: Actinomycete-feed, Treatment, Fertilizer
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The plates containing poultry feces-extract agar medium (1000g of poultry feces was added to 1000 m/ of tap water, left to stand for 10 min at room temperature, filtered through a sheet of gauze, supplement with 1.5% agar, and then adjusted to pH 8.5 with 2 N Na_2CO_3 before sterilization at 121°C for 30 min) were inoculated with diluted suspensions of various samples of manure and composted livestock feces, and followed by incubation at 50°C for 5 days.

Preparation of strain CH-53 feed additives

Solid wheat bran medium (1000g poultry feces, 1000 m/ tap water, 1000g wheat bran and 20g Ca $(OH)_2$) was dispensed in 50g amounts to 500 m/ Elrenmeyer flasks and autoclaved at 121°C for 30 min. A culture slant of the isolated strain CH-53 was inoculated to the medium and cultivated at 50°C for 14 days. After 14 days, matured spore of the strain CH-53 was air-dried at 45°C to be less than 10% moisture content and then mixed with commercial assorted feed. Dose schedule and prescription for poultry shown in Table 1.

Composting process of poultry feces

About 5 kg of excreted feces by the poultry fed with actinomycetes-feed additive and assorted feeds were inoculated on air-dried poultry feces 1 kg and $Ca(OH)_2$ 90g, at moisture content 60%, without sterilization and any additives in a plastic shallow pan and incubated at 50°C for 20 days. The treated feces were used as a fertilizer.

General analytical methods

Moisture content was measured with Moisture meter (Kett Co, Ltd). pH was measured with pH meter model HM-5B (TOA Electronics Ltd). The viable counts of the actinomycetes and coliform bacteria were determined in the poultry feces-extract and deoxycholate agar medium according to the plate dilution method, respectively. The contents of total nitrogen and organic carbon were determined by means of dry combustion a Yanaco CN-corder (MT-500). NH_4-N, NO_3-N, P_2O_5, K_2O, CEC, crude ash and SiO_2 were determined according to the soil and plant analytical method (20).

Gas chromatograph

Volatile fatty acids, which are the main compounds of the malodour of poultry feces, were analyzed quantitatively by gas chromatograph. The fresh poultry feces (50g wet matter) and the treated poultry feces by the isolated strain CH-53 (50g wet matter) were mixed with 50 m/ of distilled water, respectively, and were acidified to pH 2 with 2 N H_2SO_4. Volatile fatty acids of each samples were collected in a 50 m/ volumetric flask by distillation and their amounts were estimated by gas chromatograph at once. Hitach model 163 gas chromotograph with flame ionization detectors was used in the experiment. A glass column (2 m × 3 mm) was packed with KOCL-3000 T 1% Greensorb F40/60, Yanoco. Nitrogen was used as a carrier gas at a rate of 30 m//min. The column oven temperature was kept at 190°C and the injection port was kept at 210°C.

Pot experiments

To assess the effectiveness of treated feces as fertilizer, pot experiments were carried out in triplicated. The soil was passed through a 16 mesh sieve, and the pH was adjusted to pH 6.0 with $CaCO_3$. A total of 600g soil per pot was supplemented with the treated poultry feces, air-dried poultry feces, rapeseed meal and ammonium sulfate to give nitrogen contents of 0.1, 0.2, 0.4 and 0.8g per pot. Twenty seed of chiness mustared (*Brassica rapa* var. *perviridis*) was placed on the soil surface. Water was supplied every day to keep the 60% field capacity content. The plants were

Table 1. Dose schedule of feed containing actinomycetes to poultry

Conditions	Dose		No. of actinomycetes/g of feeds
	fed at 9 a.m	fed at 3 p.m	
Assorted feeds	40g	40g	0
Wheat bran culture of actinomycete strain CH-53	0.4g	0.4g	2.4×10^{10}
Tap water	5 m*l*	5 m*l*	0

grown at 25°C in the greenhouse. After 30 days, they were harvested and their fresh weights were measured. The fertilizer effect was expressed at the average fresh weights of the plant.

Results

Selection of actinomycetes useful for composting of poultry feces

Not less than a thousand strains of a actinomycetes isolates were collected from the colonies on poultry feces-extract agar medium. The isolated strains were treated for whether or not each of them could predominantly grow on sliced fresh poultry feces. Above all, the strain CH-53 showed aboundant growth on fresh poultry feces.

Viable count of strain CH-53 in excreted feces

Spores of strain CH-53 were fed to poultry as feed additives. After 6 days, the cell number of the actinomycetes in the excreted poultry feces increased to 10^7, and the cell number remained almost constant for about the next 18 days. On the 24th days, we stopped supplying the actinomycetes-feed additives to assortd feed as feed additive, and the population of actinomycetes immediated decreased to 10^3 (Fig. 1).

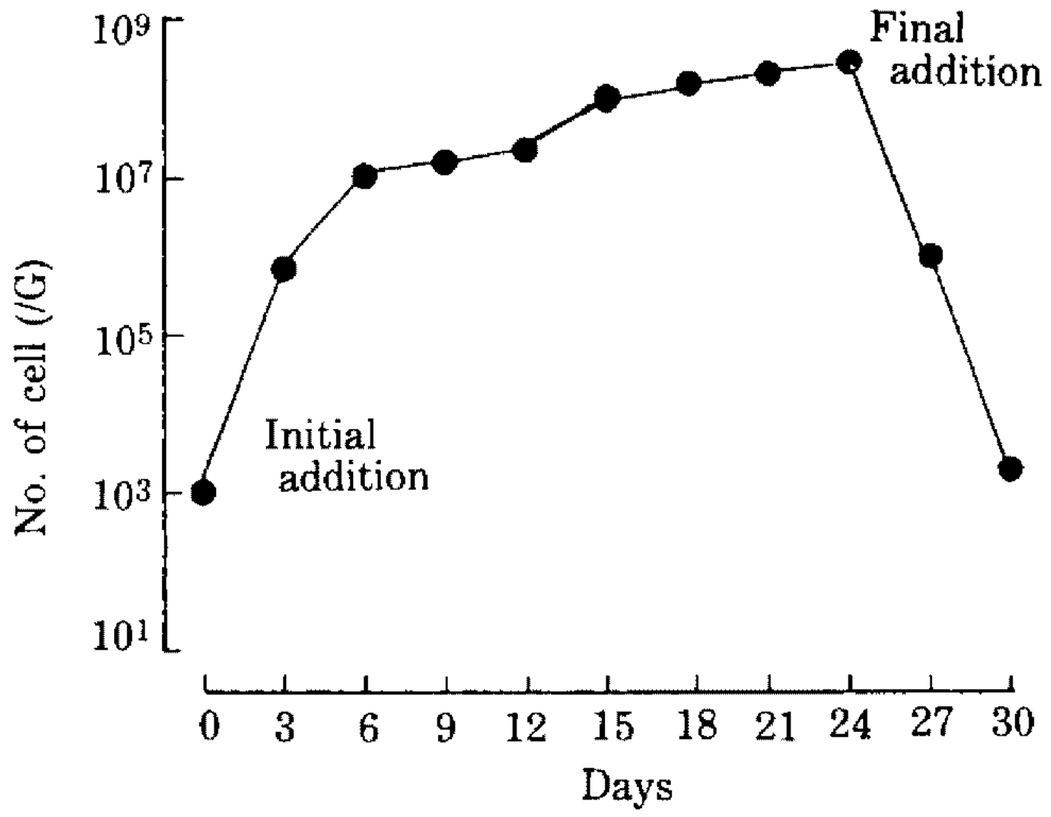

Fig. 1. Viable count of actinomycete in excreted feces.

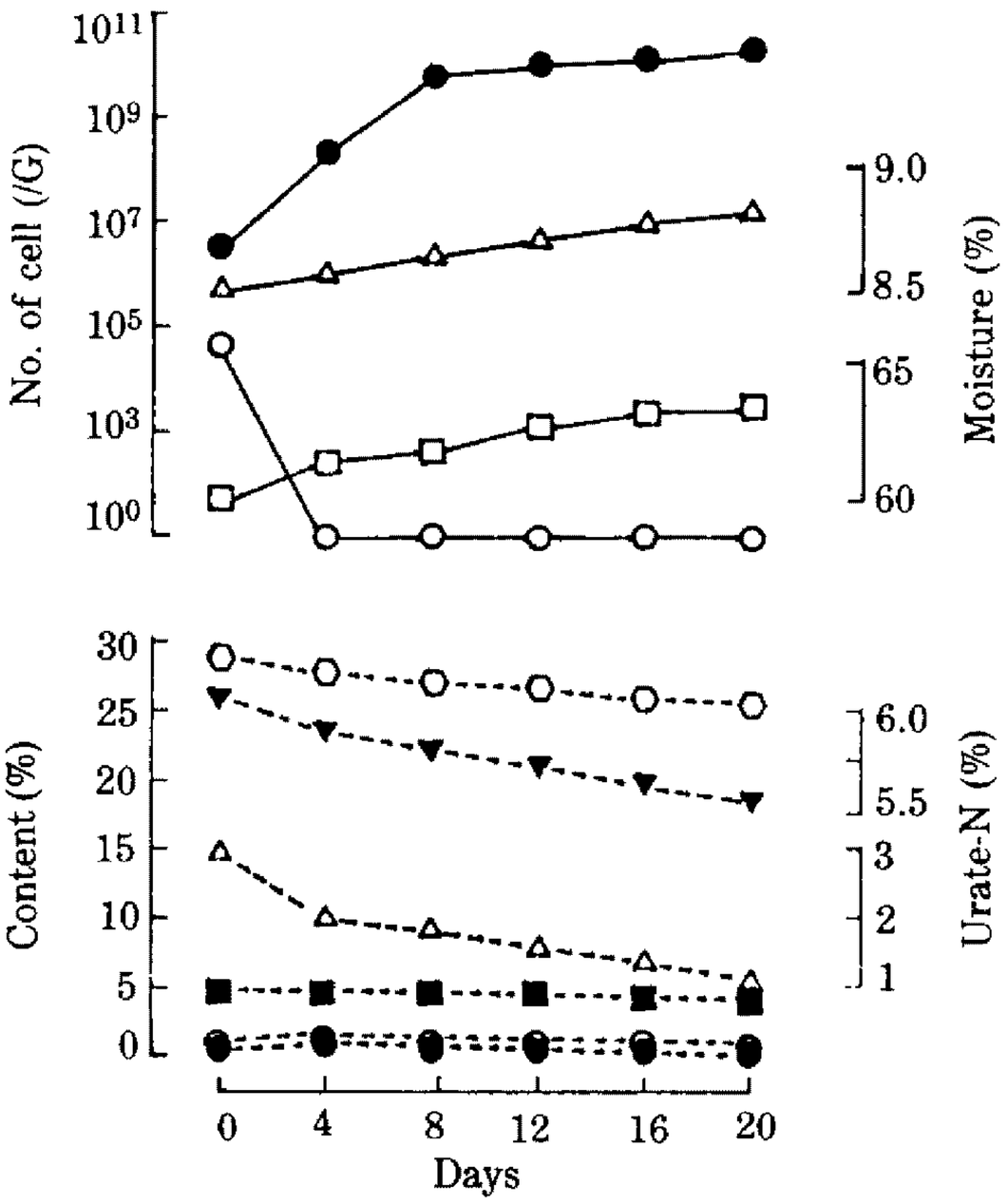

Fig. 2. Changes in microbial population, moisture and chemical composition during poultry feces treatment.
Symbols: actinomycetes (●), coliform bacteria (○), pH (△), moisture (□), total organic-C (-○-), total-N (-■-), C/N (-▼-), urate-N (△), NH_4-N (-○-), and NO_3-N (-●-).

Composting process of poultry feces

The changes in the microbial population, moisture and chemical composition during poultry feces treatment are shown in Fig. 2. The viable count of actinomycetes increased from 8×10^6/g wet matter to 2×10^9/g within 8 days. After 20 days, the final viable count of actinomycetes was 6×10^9/g wet matter. On the other hand, the viable count of coliform bacteria decreased to the extent of 10^0/g wet matter after 4 days. The contents of total organic carbon and total nitrogen decreased from 29.8% to 25.3% and 4.9% to 4.4% as dry matter during the process, respectively. The composition of the product is shown in Table 2.

Change of volatile fatty acid

The treated poultry feces and the fresh poultry feces were subject to gas chromatograph. The results are shown in Table 3. The volatile fatty acids, which are the main compounds of the malodour of poultry feces, diminished owing to utilization by the *Thermoactinomyces* sp. CH-53. Among the volatile fatty acids, the acetic acid contained in large amount in fresh poultry feces decreased 92% after treatment.

Pot experiments

The relationship between the growth conditions for chiness mustared (*Brassica rapa* var. *perviridis*) and amount of nitrogen applied with the samples in shown

Table 2. Composition of the strain CH-53 product

Total organic-C (%)	25.32
Total-N (%)	4.40
K_2O (%)	2.93
P_2O_5 (%)	5.95
Crude ash (%)	39.48
SiO_2(%)	0.91
pH value (H_2O)	8.70
C/N ratio	5.75
CEC (mg/100g)	55.45

(dry matter)

Table 3. Changes in volatile fatty acid content during poultry feces treatment

Feces	Concentration (mg/g poultry feces)						
	C_2	C_3	C_4	iC_5	C_5	iC_6	C_6
Fresh poultry feces	13.5	16.8	13.7	7.5	4.0	0.49	0.17
Treated poultry feces	1.3	0.4	0.2	0.7	0.1	0.06	0.08

C_2; Acetic acid, C_3; Propionic acid, C_4; *n*-Butyric acid, iC_5; iso-Valeric acid C_5; *n*-Valeric acid, iC_6; iso-Caproic acid, C_6; n-Caproic acid

in Fig. 3. When a series of plant cultures was fertilized with ammonium sulfate inhibition by nitrogen was observed at a nitrogen content of 0.4g N/600g soil/pot. In the case of treated poultry feces, growth inhibition caused by excess nitrogen was not observed at a nitrogen content of 0.4g N/600g soil/pot. The yield of the plants with the treated poultry feces increased even at the highest nitrogen content of 0.4g N/600g soil/pot, therefore growth inhibition was observed at a nitrogen content of 0.8g N/600g soil/pot.

Discussion

In order to treat the feces more simply and rapidly, we aimed at the use of the spores of actinomycetes as additives to commercial feed and them directly to poultry.

We tried to treat poultry feces with newly isolated thermophilic actinomycetes strain CH-53. This strain, with a growing ability in alkaline conditions and on unsterilized fresh poultry feces without any additives, utilizing ability of volatile fatty acids and decomposing activity of uric acid, was identified as *Thermoactinomyces* sp. CH-53 according to the Bergey's Mannual of Determinative Bacteriology.

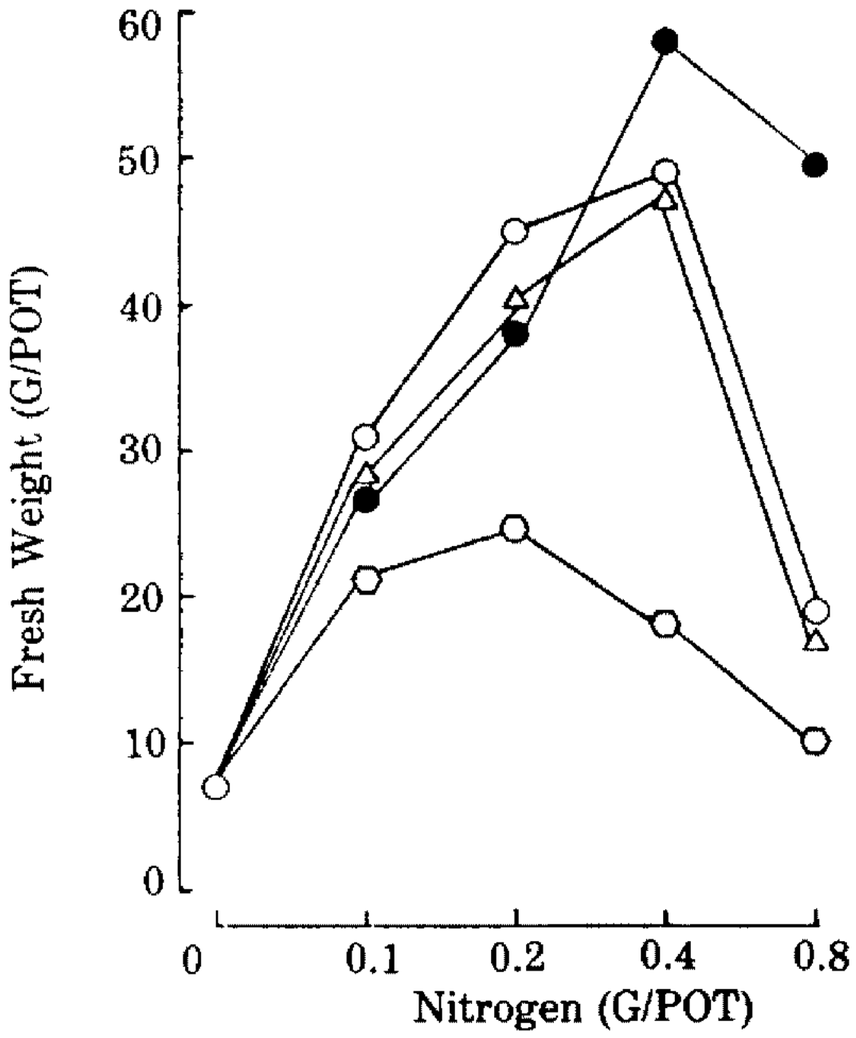

Fig. 3 Fresh-weight yields of *Brassica rapa* var. *perviridis* fertilized by treated poultry feces (●), air-dried poultry feces (△), rapeseed meal (○) or ammonium sulfate (○).

The feces excreted by poultry contained the actinomycetes spores and were deodorized very rapidly without inoculation of procedure of feces will by epochally simplified and will rid of poultry feces malódour.

After treatment, the total organic carbon content and the total nitrogen content decreased. Golueke (21) described that damage may arise from too high a C/N ratio or from the production of ammonia in the soil. If the C/N ratio applied to the soil is too high, nitrogen starvation of the crop plant may occur. Ammonia in more than trace amount has been described as toxic to plant roots (22). Moreover, it has been point out that mixing immature compost with soil will inhibit seed germination due to the porduction of toxic metabolites in an anaerobic environment (23). It is thus necessary to determine whether or not the compost has been sufficiently matured. In general, it is found that the C/N ratio of the compost declines as the treatment process progresses. In our experiment similar results were also observed.

According to the results of the pot experiment on *Brassica rapa* var. *perviridis*, the plant showed the highest yield when such a large amount of treated poultry feces as 0.4g N/600g soil/pot were supplemented as fertilizer. The highest yield of the plant with air-dried poultry feces and rapeseed meal were

shown at 0.4g N/600g soil/pot, but lower than that with treated poultry feces at 0.8g N/600g soil/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 treated poultry feces could be as an excellent fertilizer and soil improving agent.

요 약

방선균의 한 종인 *Thermoactinomyces* sp. CH-53이 퇴비에서 분리되었다. 분리된 이 균은 pH 6.5-9.5, 수분 55-65%의 무살균계분에서 왕성히 성장하며, 밀기울 배지에 포자를 충분히 착생시켜 시판의 배합사료에 1% 비율로 첨가하여 닭에 급여해서 방선균의 생균수가 10^7-10^8 cell/g에 도달하는 계분을 얻었으며, 처리 동안 악취성분이 소실되었다. 처리된 계분의 비료효과실험은 pot에서 *Brassica rapa* var. *perviridis*의 생육조사에서 다량시용에도 생육저해가 없었으며 질소함량으로 pot당 0.4g에 해당하는 시용구에서 최대생산을 보였다.

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(Received May 11, 1990)