J. Korean Agric. Chem. Soc.
한국농화학회지 31(1), 100—105 (1988)

Production of Biofertilizer from the Rice Straw Mixed with Hen Feces with *Thermoactinomycetes vulgaris*

Moo Young Choi, Shin Jyung Kang and Jae Sung Lee*

Department of Agricultural Chemistry, Faculty of Agriculture, Higashiku, Fukuoka, Japan; *Department of Food Science and Technology, Young Nam University, Kyungpook, Korea.

볏짚과 鷄糞의 혼합물로 부터 *Thermoactinomycetes vulgaris*에 의한 生物肥料의 製調

崔武永·姜信正·李在成*

日本 九州大學 農學部 農藝化學科, *嶺南大學校 食品加工學科

초 록

鷄糞과 볏짚 혼합물을 *Thermoactinomycetes vulgaris*로 처리하므로써 악취가 나지 않고 作物生育을 촉진시키는 生物肥料를 제조하였다. 肥料 製調의 처리조건은 온도 50°C pH 8.0~8.5, 水分含量 60% 였으며 醱酵 2日 후에는 악취 성분으로 알려진 휘발성 지방산이 사라졌으며 결과적으로 鷄糞의 악취가 제거되었다. 製調된 肥料의 肥效實驗은 pot에서 *Brassica rapa* var. *previdis*의 生育을 調査함으로서 행하였다. 生物肥料는 황산암모니아, 건조鷄糞, 평지깻묵 보다 作物生育을 촉진 시켰으며 多量의 試用으로도 生物의 生育저해가 작았다. 질소함량으로 pot當 0.8g에 해당하는 生物肥料를 含有하는 試用區에서 作物의 最大生産量을 보였다.

Introduction

In recent years, due to raising a large number of domestic animals in one place, large amounts of the excrement produced by them has polluted environment. The application of livestock wastes to agricultural land as a fertilizer could become one of valuable means for preventing environmental pollution. Animal excrements, especially hen feces, are usually treated by using activated sludge method or are made into a dry manure by sundring. These methods have a demerit because of exhalation of malodor during treatment.[8] Some scientists[2,3,4,7] have studied on the treatment of hen feces by using solid state fermentation equipment. But these treatment methods also require a large scale plant, high costs, and long treatment time. Accordingly, more economical and sanitary treatment method should be studied for making animal excrements into a good manure.

This paper describes the production of "biofertilizer" from the rice straw added to unsterilized hen feces by using *Thermoactinomycetes vulgaris* and the growth promoting effects of the biofertilizer on plant.

Received January 12, 1988
Corresponding author: S.J. Kang
Present address of S.J. Kang: Department of Agricultural Chemistry, Kyungpook National University, Taegu, 635, Korea.

meterials and Methods

Organism

Thermoactinomycetes vulgaris(IFO 14051) was obtained from Institute for Fermentation Osaka. The growth under anaerobic condition was chec-

ked on hen feces extract agar using Gas-Pak Anaerobic Systems(Becton Dickenson Co.). This strain, however, grow vigorously in aerobic conditions(Fig. 1).

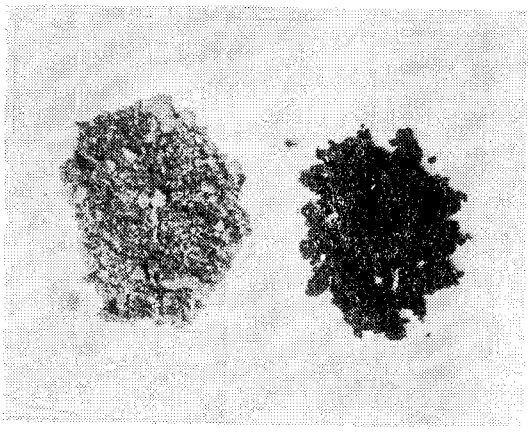

Fig. 1. Hen feces treated with *Thermoactinomycetes vulgaris*
A : 48h. B : 0h.

Production of biofertilizer

The *Thermoactinomycetes vulgaris* was inoculated on a slant of hen feces extract agar medium (1000g of hen feces was added to 1000ml of tap water and left to stand for 10min. at room temperature. After filtration through a sheet of gauze, 1.5% of agar was added and pH was adjusted to 8.5 with 2N-Na_2CO_3 before sterilization at 121°C for 30min.) and incubated at 50°C for 7 days. The spores were collected, inoculated to wheat bran medium(25g wheat bran, 27ml hen feces extract, 0.8g $Ca(OH)_2$ and moisture content 60%) in 500ml Erlenmyer flask and incubated at 50°C for 2 weeks for seed culture. The seed (1×10^{13} cells/g) was inoculated to the extent of 5% into unsterilized raw material mixture that is consisted of fresh hen feces 5kg, rice straw (air-dried and crushed into about 1mm pieces in diameter) 1kg, $Ca(OH)_2$ 90g and moisture content of about 60% in a shallow aluminum pan. After 20 days of incubation at 50°C, it was used as a "biofertilizer."

Composition of biofertilizer

Moisture content of biofertilizer during its fermentation was measured with moisture meter (Kett CO. Ltd.) every day and was controlled to about 60% through the addition of water. For pH, 1g of sample was suspended in 10ml of distilled water, kept for 10min. at room temperature and then measured with pH meter(Model HM-5B, TOA Electronics Ltd.). The viable counts of the actinomycetes and *Escherichia coli* were made on hen feces extract agar medium and desoxycholate agar medium according to the plate dilution method, respectively. The contents of total nitrogen and organic carbon were determined by dry combustion using a Yanaco CN corder(MT 500). NH_4-N, NO_3-N, P_2O_5, K_2O, CEC, crude ash, and SiO_2 were measured according to the soil and plant analytical method.[1)]

Gas chromatography

Ten g of hen feces were acidified with 1N-HCl and extracted with CH_2Cl_2 for 3 hours. The extract was condensed to 10ml for gas chromatography after removing water with Na_2SO_4. Sample solution was analyzed with Hitachi Model 163 Gas Chromatograph equipped with a flame ionization detector. A glass column was packed with DEGS+H_3PO_4(2%+5%)/Chromosorb WA W. Nitrogen was used as a carrier gas at a rate of 30ml/min. The column oven and injection port temperature was kept at 80°C and 150°C, respectively.

Pot experiment

Humic volcanic ash soil from Kuroishibaru, Kumamoto prefecture in Japan was used. The pH value of the soil was adjusted to 7.0 with lime and the moisture content was maintained at about 60% throughout the experiment. Biofertilizer, air-dried hen feces, rapeseed meal, and ammonium sulfate were used as fertilizers. Each fertilizer was added to soil in such a way that nitrogen content would be 0.0, 0.1, 0.2, 0.4, 0.8, and 1.6g N/pot. One pot contained 600g soil. *Brassica rapa* var. *previdis* was planted on December 1st. and harvested on December 30th in 1986. The

experiment was carried out in triplicate. The twenty-five seeds of *Brassica rapa* var. *previdis* were sprinkled uniformly. Random rotation of the pots were also carried out every day for minimizing the influence of location. All pots were cultivated at 25°C and 70% humidity in a thermostatically controlled greenhouse. Fresh weights of plants were measured after 30 days of cultivation.

Nitrogen degradation

Biofertilizer, air-dried hen feces, rapeseed meal, and ammonium sulfate were employed in this experiment. The samples were air-dried and crushed into pieces below 1mm in diameter before use. The amount of each samples containing 20g of nitrogen content were mixed with 100g of soil in 100ml Erlenmeyer flasks, respectively. The flasks were covered with ventilative vinyl and incubated at 30°C for 4 weeks. At suitable interval, inorganic nitrogen was measured according to the Conwey method.

Results

The production of biofertilizer

The changes in moisture content, microbial population and chemical composition during hen feces treatment are shown in Fig. 2. Moisture content was maintained at about 60%. The viable counts of the actinomycetes increased from 4×10^6/g to 2×10^{10}/g within 8 days. After 20 days, the final viable counts of actinomycetes was 4.6×10^{11}/g. On the other hand, the viable counts of *Escherichia coli* decreased to the extent of 10^0/g after 4 days. The pH increased from 8.0 to 9.2 during treatment. The contents of total organic carbon and total nitrogen decreased gradually during treatment. C/N ratio decreased from about 10 to 6.7. The composition of biofertilizer manufactured is shown in table 1. The malodor components, volatile fatty acids,[5,6] of the hen feces disappeared after two days of fermentation in biofertilizer manufacturing process(Fig. 3).

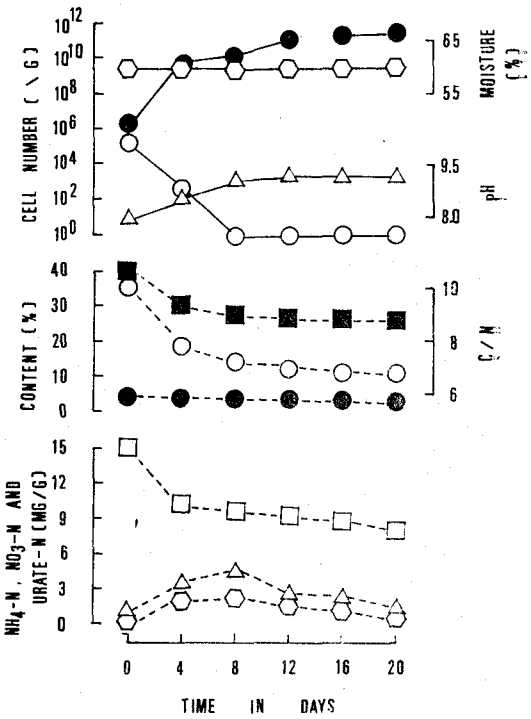

Fig. 2. The changes in moisture content microbial population and chemical composition during hen feces treatment
● ; *Thermoactinomycetes vulgaris.* ⬠ ; Moisture. △ ; pH. ○ ; *Escherichia coli.* —■—; Total organic carbon. —●— ; Total nitrogen. —○— ; C/N-ratio. —□— ; Urate-N. —△— ; NH_4-N. —⬠— ; NO_3-N.

The effect of biofertilizer on plant growth

The relationship between growth and the amount of nitrogen supplied by the samples are

Table 1. Composition of biofertilizer

Total-N (%)	Total organic-C (%)	C/N	pH (H_2O)	K_2O (%)	P_2O_5 (%)	NH_4-N (mg/g)	NO_3-N (mg/g)	CEC (me/100g)	Crude ash (%)	SiO_2 (%)
3.87	26.28	6.7	9.2	2.85	3.05	2.19	1.58	49.25	35.11	10.67

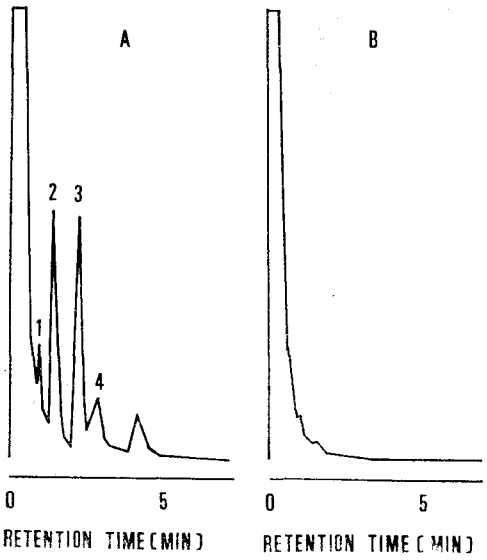

Fig. 3. Gas chromatography of volatile fatty acids A : fresh hen feces B : treated hen feces. 1. Acetic acid; 2. propionic acid; 3. n-butyric acid; 4. isovaleric acid. Condition: Sample size(methylated), 0.2μl, column; chromosorb W AW(DEGS+H_3PO_4(2%+0.5%), detector; flame ionization detector, Carrier gas; N_2(30 ml/min), column temperature; 80°C, injection temperature; 150°C.

shown in Fig. 4 and 5. Ammonium sulfate and air-dried hen feces showed promotion effect on plant growth at 0.1 and 0.2g N/pot, respectively, but inhibition of plant growth were observed at the nitrogen content over 0.2 and 0.4g N/pot, respectively. When ammonium sulfate and air-dried hen feces were given to the level of 1.6g N/pot, no growth of the plant was observed. Biofertilizer and rapeseed meal showed the best promotion effect on plant growth, without growth inhibition, at the pot of 0.8g N/pot.

Mineralization of organic nitrogen

The degradation rate of nitrogen in each sample is shown in Fig. 6. Air-dried hen feces showed more than 90% of degradation rate in a week. However, biofertilizer indicated the slowest degradation and had a tendency of degradation even after 4 weeks.

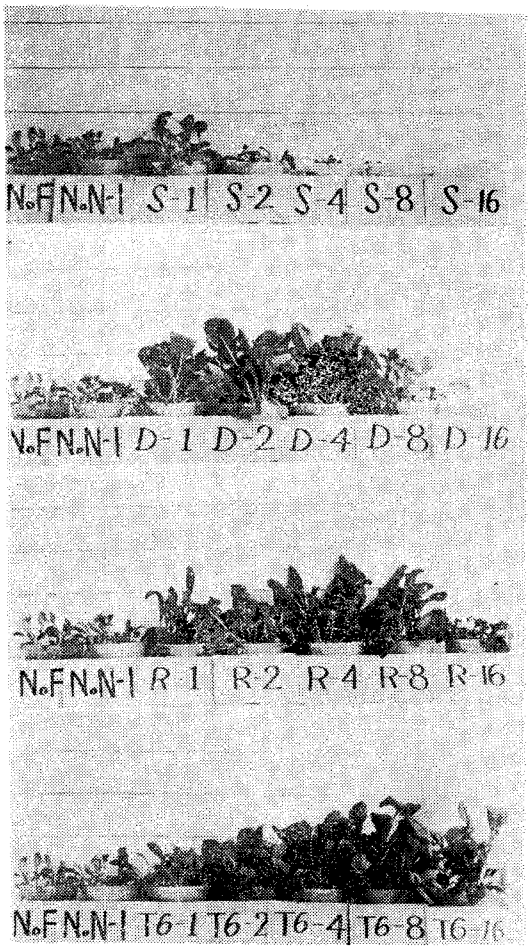

Fig. 4. Growth of *Brassica rapa* var. *previdis* R : Rapeseed meal. S : Ammonium sulfate. T_6 : Treated hen feces(biofertilizer). D : Air-dried hen feces. N_0F : Without fertilizer. N_0 N-1 : Without nitrogen. numbers are given as $(10\times\frac{gN}{pot})$.

Discussion

In order to establish the method of livestock wastes utilization, hen feces mixed with rice straw was treated with *Thermoactinomycetes vulgaris* IFO 14051. The strain showed the vigorous growth on the rice straw added into unsterilized hen feces at the moisture content of 60% and pH 8.0~9.0 under which the conditions the growth of anaerobic bacteria was inhibited. Especially, the *Thermoactinomycetes vulgaris* preferentially grew on the feces extract media, so it belongs to

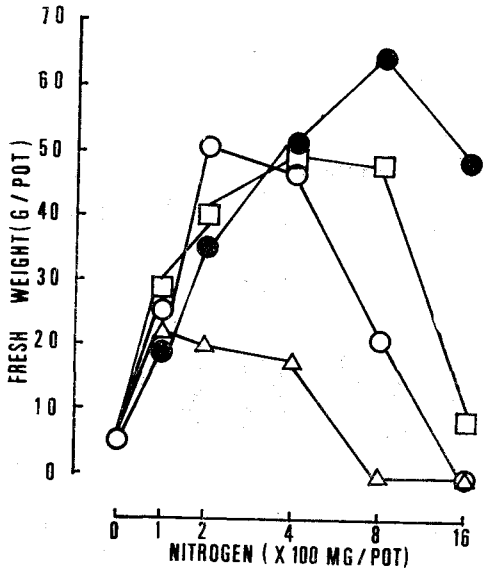

Fig. 5. Fresh weight yields of *Brassica rapa* var. previdis
● ; Treated hen feces(biofertilizer). ○ ; Air-dried hen feces. □ ; Rapeseed meal. △ ; Ammonium sulfate.

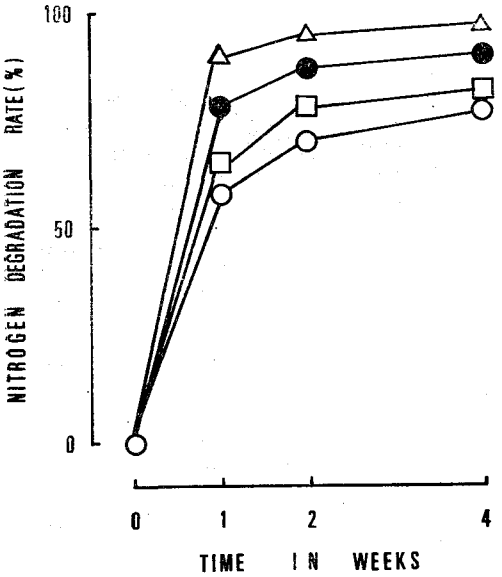

Fig. 6. Nitrogen degradation rate of each sample
○ ; Treated hen feces(biofertilizer). △ ; Air-dried hen feces. □ ; Rapeseed meal. ● ; Ammonium sulfate.

a group of the coprophilous microorganisms. In the production process of biofertilizer, the nitrogen content decreased gradually during treatment. This might be due to the generation of ammonia by microbial degradation, followed by the escape from the system. The biofertilizer containing nitrogen as microbial mycelium was superior to chemical fertilizer.

Low molecular weight fatty acids, which cause the specific malodors of the feces, were not detected in the feces treated with *Thermoactinomycetes vulgaris* as shown in Fig. 3. These fatty acids might have been metabolized by the *Thermoactinomycetes vulgaris* and/or lost into the air.

According to the pot experiment with *Brassica rapa* var. *previdis*, the plant showed the highest yield, without growth inhibition, even when such a large amount of biofertilizer as 0.8g N/pot was supplemented as fertilizer. The highest yield of the plant with ammonium sulfate, air-dried hen feces, and rapeseed meal was shown at 0.1, 0.2 and 0.8g N/pot, respectively, but lower than that with biofertilizer at 0.8g N/pot(Fig. 4 and 5). The reason for these results was presumed to be that biofertilizer contains most nitrogen as microbial mycelia, and those mycelia are gradually decomposed and continually supply the plant with proper amount of nitrogen.

It is suggested that biofertilizer could be used as the excellent fertilizer and soil improving agent.

Abstract

A biofertilizer, having been deordorized and showing promotive effect on plant growth, was manufactured from the rice straw and hen feces by use of *Thermoactinomycetes vulgaris*. This strain grew vigorously on rice straw mixed with unsterilized hen feces at 50°C, pH 8.0~8.5 and moisture content of 60% and got rid hen feces of malodour during treatment. The growth of plant(*Brassica rapa* var. *previdis*) was experimented on humic volcanic ash soil, using pot in thermostatically controlled greenhouse. The biofertilizer was applied as N-fertilizer and air-dried hen feces or ammonium sulfate were used for comparison with the biofertilizer. The effect on plant growth was evaluated on the basis of the

amount of nitrogen as fertilizer, under a loading of 0.1g N/pot, all samples showed a promotion effect of plant growth. But ammonium sulfate and air-dried hen feces inhibited plant growth at the nitrogen content over 0.2 and 0.4g N/pot, respectively, whereas the biofertilizer showed a good promotion effect on plant growth without growth inhibition even at nitrogen content of 0.8g N/pot.

References

1. Aomine, S., and Harada, T.: Dojo hiro gaku jikken note (soil and plant analytical method). Publishing Co. Yokendo(1966)
2. Fujio, Y., Ohtomi, Y., Hayashi, S. and Ueda, S.: J. Ferment. Technol., 56 : 304(1978)
3. Fujio, Y., Ohotomi, Y. and Ueda, S.: J. Ferment. Technol., 58 : 17(1980)
4. Hayashida, S., and Tanaka, Y.: Seibutsu niyoru kankyo jouka. Ed. by K. Arima and G. Tamura. Gakka shuppan center, Tokyo. p. 243～250(1980)
5. Miner, J.R.: Agric. Environm., 3 : 129(1977)
6. Roustan, J.L., Aumaitre, A. and Salmon-Legagneur, E.: Agric. Environm., 3 : 147(1977)
7. Tanaka, Y., Hayashida, S. and Hongo, M.: J. Ferment. Technol., 55 : 134(1977)
8. Watanabe, T.: Anim. Husbandry, 30 : 119 (1970)