Effect of Lactic Acid Fermentation Bacteria on Plant Growth and Soil Humus Formation

T. Higa and S. Kinjo
University of The Ryukyus, Okinawa, Japan

Abstract
A study was conducted to determine if lactic acid bacteria, when inoculated into soil amended with organic materials, could enhance decomposition and the release of plant nutrients, and increase soil humus formation. The addition of EM 4 to soil amended with fresh green grass increased the growth of cucumber over that of the unamended and fertilized controls, while woodchips appeared to suppress growth. The yield of mustard and radish (tops and roots) were significantly higher with EM 4 at all dilution levels than either of the controls. However, mustard yield was highest at the 1:500 dilution, while there was little difference in radish yield for the dilutions used. Populations of fungi, lactobacilli, aerobic bacteria, and actinomycetes were generally higher in soil treated with EM 4 than for the unamended controls. Results indicate that EM 4 can accelerate the decomposition of organic amendments in soils and the release of their nutrients for plant growth. The soil humus content, even from addition of woodchips, was increased considerably from treatment with EM 4.

Introduction

Decomposition of organic matter in soil results in gas and heat production which are lost energy to the cultivated crop. This kind of decomposition can result in products that are harmful to plant growth (Gussin and Lynch, 1981). Nutrient recycling in terrestrial environments would be more efficient if this energy loss could be avoided. Fermentation pathways provide a more efficient means for utilizing organic substrates during their decomposition in soil.

Fermentation is a process that is often used in producing and preserving foods and beverages such as yogurt, cheese, and beer. In Japan, soy sauce (shouyu) and soybean paste (miso) are just two of many examples of fermentation. The fermentation process not only preserves foods from spoilage, but may even increase the nutrient value of the product.

Lactic acid fermentation bacteria are also responsible for the preservation of vegetable products for human consumption and silage for animals. There is some indication that lactic acid can inhibit the growth of microorganisms that are responsible for spoilage (Lynch, 1988). Nevertheless, high concentrations of lactic acid can provide good preservation (Langston and Bouma, 1960). Lactic acid bacteria have also been used for treatment of cattle manures and sewage for odor abatement, and as an inoculant to accelerate the composting of organic wastes (Okada, 1988).

The possibility of using this fermentation process in a soil environment has been of great interest, When green plant material is incorporated into the soil there is little loss of energy as gas and heat, and the phytotoxic effect that usually follows the decomposition of plant materials is minimized (Lynch, 1977).

The purpose of this study was to determine whether lactic acid bacteria inoculated into soil amended with different organic materials as substrates, could provide a more effective means for recycling plant nutrients and for increasing soil humus formation.

Materials and Methods

Experiment 1

A greenhouse pot experiment was conducted in October 1987 to determine the effect of EM on plant growth and soil humus formation. Soil was mixed with chopped crabgrass (*Digitaria ascendens* Henri) or woodchips at rates of 0, 0.5, 2.0, and 4.0 Mg 10 are^{-1} (10 are is 0.1 hectare), i.e., 0, 5, 20, and 40 Mg ha^{-1}, and placed in vinyl pots (20 cm diameter and 25 cm depth). Cucumber seeds were sown and EM 4 was applied to the soil each week at dilutions of 1:500, 1:1000, and 1:2000. EM 4 is comprised mainly (more than 90 %) of lactobacillus bacteria, but also contains photosynthetic bacteria, ray fungi, and yeasts. During the initial growth period, each pot received 45 mg of nitrogen, 25 mg of phosphoric acid, and 51 mg of potassium. Plant growth was recorded four times and soil samples were collected three times for analysis of NO_3^-, NH_4^+, pH, P_2O_5, K_2O, and humus.

Experiment 2

A greenhouse pot experiment was conducted in November 1988 in much the same manner as Experiment 1, except green (fresh) chopped rose grass was mixed with soil at a rate of 40 Mg ha^{-1}. Mustard seeds were then sown at 0, 1, 2, 3, and 5 weeks after addition of the organic amendment. A culture of *lactobacillus plantarum* was applied to the pots each week at dilutions of 1:500, 1:1000, and 1:2000.

Lactobacillus plantarum (IFO 3070) was cultured at 30^oC in GYP + 1 % skim milk liquid medium (Okada, 1988); 1 ml of the culture contained approximately 1.2 x 10^9 bacteria. The culture was suspended in distilled water at a dilution of 1:10 and then further diluted, as previously described, for application to soil. Both fresh and dry weight crop yields were recorded. Soil microorganisms were enumerated by the plate count method using modified GYP agar (Okada, 1988) for lactobacilli; egg albumin agar (Tadao, 1984) for aerobic bacteria; rose bengal agar (Martin, 1950) for fungi; and a selective media (KenKnight and Muncie, 1939) for actinomycetes. The soil was analyzed initially and upon completion of the experiment for pH, K_2O, P_2O_5, NO_3^-, NH_4^+, and humus content.

Experiment 2b

A second experiment was conducted in March of 1989 with the soil that had been used in Experiment 2. The potted soil was amended with fresh green plant material from wild marigold (*Wedelia trilobata* Hitchc.) at a rate of 20 Mg -1 and radish seeds were sown. The *Lactobacillus* culture was applied to the pots at the same dilutions and frequency as in Experiment 2. Methods for enumerating soil microbial populations and soil analysis were the same as in Experiment 2.

Results

Experiment 1

As shown in Table 1, amending soil with green (fresh) chopped organic material resulted in better growth (height) of cucumber plants than from incorporation of woodchips , especially at the higher application rates of 20 and 40 Mg ha^{-1}. Inoculation of soil with lactobacillus bacteria (EM 4) enhanced the growth of cucumber over that of the unamended and fertilized controls. Amending soil with woodchips appeared to suppress the growth of cucumber particularly at the highest rate of incorporation (40 Mg ha^{-1}) as shown in Tables 1 and 2.

There was little difference in the chemical analysis of soils among the treatments. However, at the end of the experiment the humus content was considerably higher in soil that had been amended with organic materials and inoculated with lactobacilli from EM 4 (Figure 1).

Table 1. Effect of Soil Organic Amendments and Different Dilutions of EM 4 on Height of Cucumber

Dilution*	Rate of Application**						
	Control	Green Grass			Woodchips		
	0	5	20	40	5	20	40
	cm	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
Control (No EM)	91	111	140	133	72	86	49
1:2000	109	131	148	153	85	83	48
1:1000	114	129	146	143	92	81	49
1:500	129	126	136	136	94	67	55

* EM 4 dilutions were made from liquid stock culture that contained 1.2 x 10^9 bacteria per ml.

** Mg ha^{-1} (5, 20, and 40 Mg ha^{-1} correspond to 0.5, 2.0, and 4.0 Mg 0.1 ha^{-1}).

Table 2. Effect of Amending Soil with Woodchips and Different Dilutions of EM 4 on Height of Cucumber.

Dilution*	Rate of Application**			
	Control	Woodchips		
	0	5	20	40
	cm	*cm*	*cm*	*cm*
Control (No EM)	123	125	162	90
1:2000	163	154	127	69
1:1000	162	144	113	64
1:500	162	152	101	71

* EM 4 dilutions were made from liquid stock culture that contained 1.2 x 10^9 bacteria per ml.
** Mg ha^{-1}

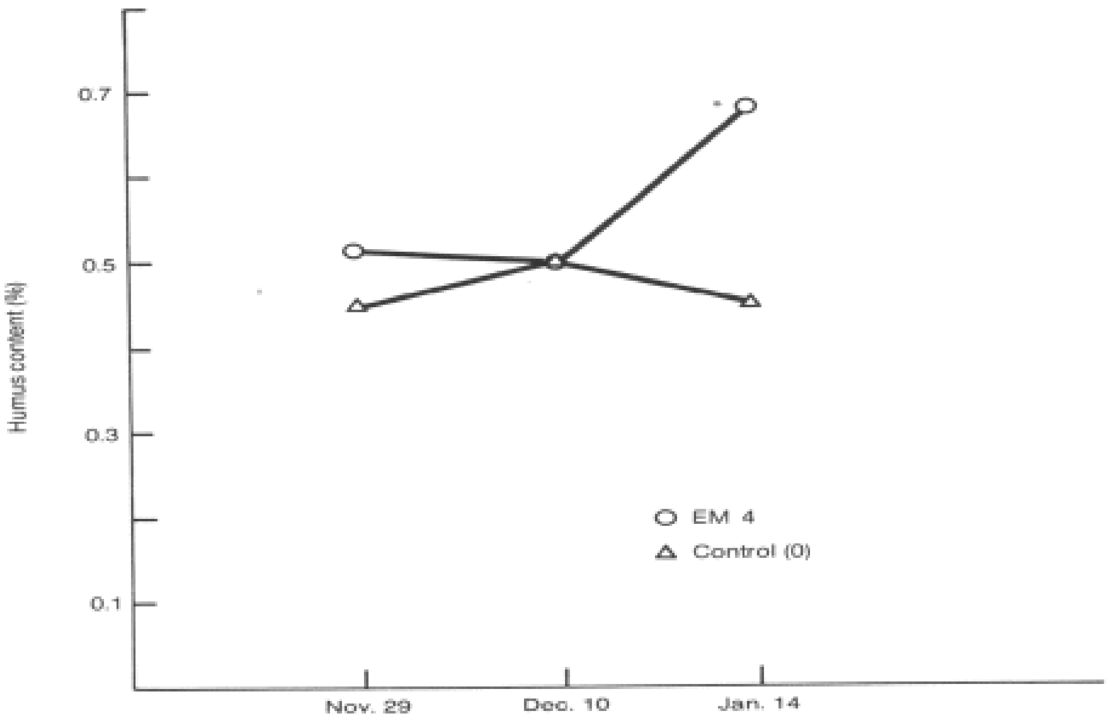

Figure 1. The Effect of Lactobacilli Inoculation the Humus Content of Soil.

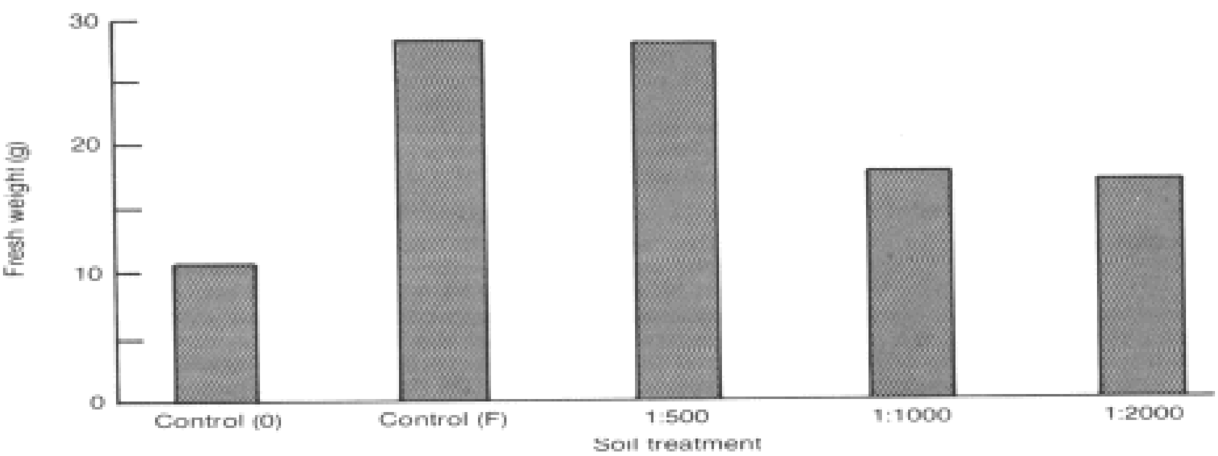

Figure 2. The Effect of Lactobacilli on the Fresh Weight Yield of Mustard Tops.

Experiment 2

Yield of mustard (biomass) was highest, and approximately the same, for the control pots that were amended with 40 Mg ha^{-1} of green (fresh) chopped rose grass, and those treated with EM 4 at a dilution of 1:500 (Figure 2). Yields from inoculation of soil with EM 4 at dilutions of 1:1000 and 1:2000 were somewhat lower.

There were no differences in the chemical analysis of soils among the various treatments. However, the numbers of microorganisms increased significantly in soil that was inoculated with EM 4 (Table 3). An exception to this was for actinomycetes which were lower in number for the 1:500 and 1:2000 EM 4 dilutions compared with the amended (and uninoculated) control.

Table 3. Effect of an Organic Amendment and Different Dilutions of EM 4 on Microbial Populations in Soil Planted to Mustard*.

Treatment**	Lactobacilli $l0^2\ g^{-1}$	Fungi $l0^4\ g^{-1}$	Aerobic Bacteria $l0^5\ g^{-1}$	Actinomycetes $l0^5\ g^{-1}$
Control (Unamended)	1.38	1.31	1.45	0.0
Control (Amended)	0.512	7.74	5.03	2.62
1:2000	1.85	2.72	5.70	0.278
1:1000	1.51	2.12	11.8	7.02
1:500	2.07	2.44	6.09	0.976

* Microbial counts were made at time of harvest and are expressed as numbers per gram of dry soil.

**EM 4 dilutions were made from liquid stock culture that contained 1.2 x 10^9 bacteria per ml. The organic amendment, green chopped rose grass, was mixed with all pots, expect the unamended control, at a rate of 40 Mg ha^{-1} (4.0 Mg 0.1 ha^{-1}).

Experiment 2b

Yield of radish tops (Figure 3) and radish roots (Figure 4) from application of lactobacilli to soil, at all three dilutions, was significantly greater than the unamended or amended controls. Again, there was little difference in soil chemical analyses due to treatments. Soil microbial populations were higher in the amended control than the unamended control (Table 4). Actinomycete populations were higher from application of lactobacilli compared with the amended control; however, the numbers of aerobic bacteria were somewhat lower than for the amended control. Fungal populations were highest after soil inoculation with lactobacilli at a dilution of 1:2000, while lactobacilli bacteria were highest at a dilution of 1:500.

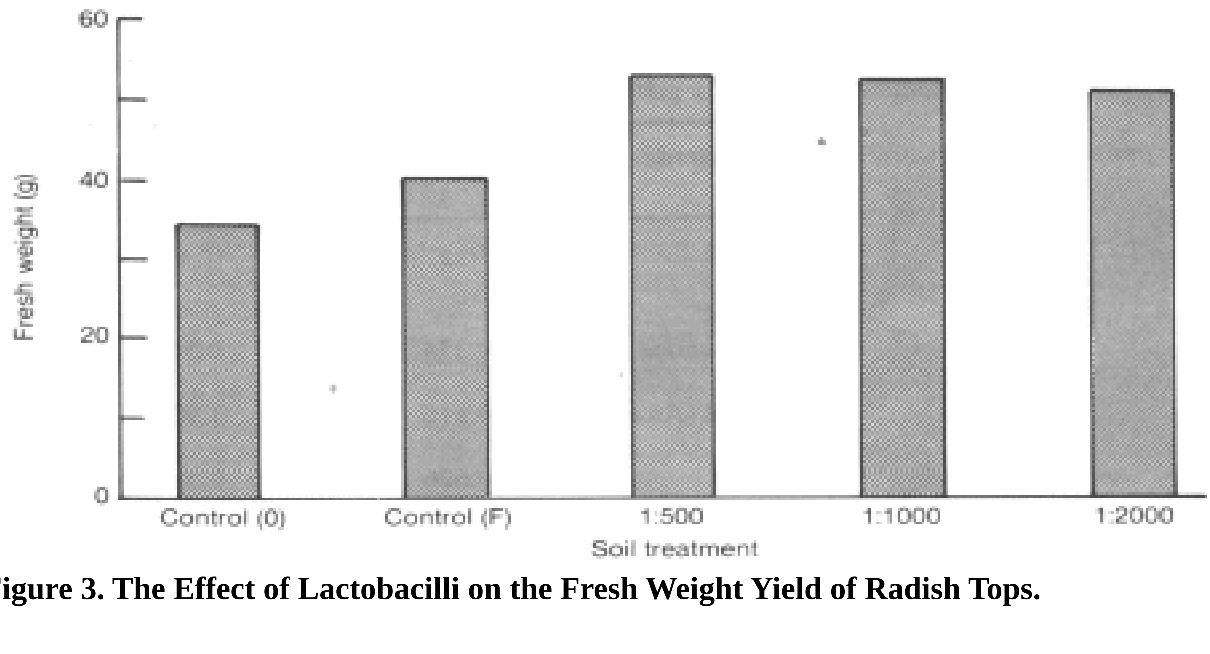

Figure 3. The Effect of Lactobacilli on the Fresh Weight Yield of Radish Tops.

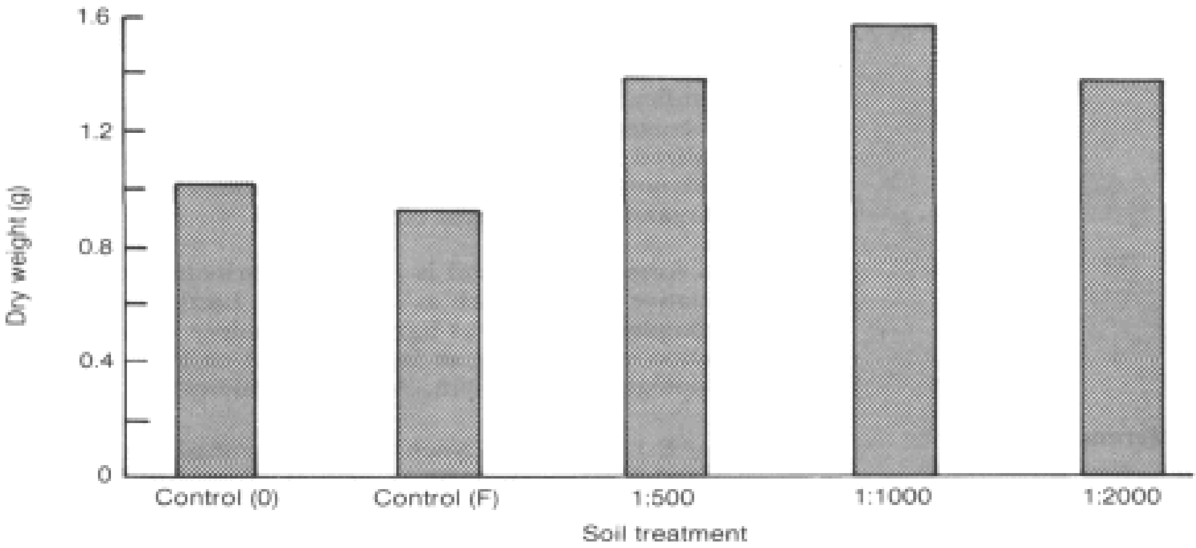

Figure 4. The Effect of Lactobacilli on the Dry Weight Yield of Radish Roots.

Table 4. Effect of an Organic Amendment and Different Dilutions of EM 4 on Microbial Populations in Soil Planted to Radish.*

Treatment**	**Lactobacilli** *$l0^2$ g^{-1}*	**Fungi** *$l0^4$ g^{-1}*	**Aerobic Bacteria** *$l0^5$ g^{-1}*	**Actinomycetes** *$l0^5$ g^{-1}*
Control (Unamended)	0.274	0.549	3.50	4.73
Control (Amended)	0.816	1.92	7.38	8.34
1:2000	0.676	2.58	5.34	10.7
1:1000	1.45	1.77	5.84	9.02
1:500	2.04	1.96	4.39	9.97

* Microbial counts were made at time of harvest and are expressed as numbers per gram of dry soil.

**EM 4 dilutions were made from liquid stock culture that contained 1.2 x 10^9 bacteria per ml. The organic amendment, green chopped wild marigold, was mixed with all pots, expect the unamended control, at a rate of 20 Mg ha^{-1} (2.0 Mg 0.1 ha^{-1}).

Discussion

In the first experiment, the incorporation of woodchips in soil resulted in poor initial growth of cucumber, possibly because of immobilization of inorganic nitrogen. The results indicate, however, that EM 4 can be used successfully in combination with woodchips as an organic amendment by accelerating their rate of decomposition in soil. This is evidenced by the fact that the soil humus content from woodchips was significantly higher at the end of the experiment due to inoculation with EM 4. In most cases, the inoculation of unamended soil with EM 4 resulted in better growth of cucumber compared with soil amended with woodchips. The enhanced growth due to EM 4 may be related to the utilization of plant root exudates (Hale et al., 1978), and the solubilization and mineralization of certain soil nutrients to plant available forms.

In the second experiment, results from the initial cropping were not conclusive. However in the second cropping, radish yields were higher from inoculation of soil with lactobacilli compared with the uninoculated controls. The higher root dry weight of radish shows the effective influence of lactobacilli on plant growth. A preliminary observation is that a more concentrated application of lactobacilli should probably be made during the initial growth period to achieve best results. Thereafter, the lactobacilli concentration can be reduced with time.

No significant differences were noted in the chemical analyses of the soil, including the humus content. A possible explanation for this may be that a pure culture of lactobacilli was utilized rather than a mixed culture. The lactobacilli are known to have a rather complex nutrient requirement and may require the presence of other compatible microorganisms to be most effective.

Humus formulation in soil is generally attributed to microorganisms other than the fermentative bacteria such as lactobacilli. Lactic acid bacteria do not readily decompose such complex materials as lignin and cellulose (Okada, 1988). Thus, it is likely that the application of EM 4 has an indirect effect on humus formation by modifying the soil microbiological equilibrium. Additional studies are needed to verify this possibility.

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