

# Effect of Microbial Fertilizers on Yield of Young Radish(*Raphanus sativus* L.)

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## 열무에 대한 土壤改良劑의 效果

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### 〈 목 차 〉

摘 要	Ⅲ. Results and Discussion
I. Introduction	IV. Absrtact
Ⅱ. Literature Review	Literature Cited
Ⅲ. Materials and Methods	

### 摘 要

本 實 驗 은 土 壤 改 良 劑 가 열무(*Raphanus sativus* L.)에 미치는 影 響 을 알 아 보 기 위 해 修 行 하였다. 土 壤 改 良 劑 로 는 MPK + Husk + Palma, Husk + Palma, MPK + Compost, Compost, Bio Livestock Cattle System(BLCS) Cattle Dropping 및 Tomi를 사용하였다.

實 驗 結 果, 모 든 微 生 物 劑 處 理 區 에 서 植 物 體 의 生 育 特 性 이 無 處 理 에 비 하여 收 量 이 增 加 하였다. 특 히, BLCS Cattle dropping 處 理 는 植 物 生 體 重, 葉 數, 및 乾 物 重 에 서 가 장 큰 效 果 를 보 였 고, MPK + Husk + Palma 는 葉 長 에 서, Husk + Palma 는 糖 度 에 서, Tomi 處 理 區 는 葉 幅 에 서 各 各 效 果 의 이 었 다.

열 무 의 化 學 成 分 에 대 한 土 壤 改 良 劑 의 效 果 는 Tomi 處 理 區 에 서 인 산 의 含 量 이 다 른 處 理 區 에 서 보 다 훨 씬 增 加 했 다. 칼 륨 의 含 量 은 모 든 處 理 區 에 서 無 處 理 區 와 비 교 하 여 高 度 의 有 意 성 을 보 였 고, Compost 處 理 區 에 서 가 장 높 았 다.

土壤의 化學成分에서는 處理間에 인산과 칼륨含量이 有意性을 보였다. 인산은 Tomi處理區에서 가장 높게 나타났고, 칼륨은 Husk + Palma處理區에서 가장 높게 나타났다.

Tomi處理區에서 인산과 칼륨의 含量은 植物體, 土壤 모두 고르게 增加를 보였다.

土壤의 微生物相을 調査한 結果, 總細菌數, *Bacillus*, 放射線菌類 및 곰팡이류가 有意性을 보였다. 總細菌數는 Tomi處理區에서, *Bacillus*군과 放射線菌類는 MPK + Husk + Palma處理區에서, 곰팡이류는 Tomi處理區에서 매우 높았으며 다른 處理間에는 差異를 보이지 않았다.

## I. Introduction

In the last few years, there have been many significant contributions in and from soil microorganisms; for example, the studies showing the crucial role of the soil microflora in modifying or destroying environmental pollutants and the investigations of the influence of the microflora on the degradation and persistence of agricultural pesticides. There has also been considerable emphasis on saprophytes and pathogens in the soil as related to plant disease.

Contemporary agriculture is severely modifying and polluting the natural environment, due to the widespread application of chemical fertilizers, herbicides and pesticides. Microbial fertilizers, considered one of the biological ways to improve crop yield, are demonstrated that they can act against detrimental microorganisms and make variable vitamins or antibiotics and enzymes etc. producing favorable microorganisms by using beneficial microorganisms to crops or domesticated animals. They are thus considered a valid alternative to chemicals. Recently as people are in favor of less contaminated crops by the widespread application of chemical fertilizers, herbicides and pesticides, and interest in environment is being increased, not only do the use of microbial fertilizers popularize, but their importance is highly focused as well.

On the other hand, rhizosphere community such as bacteria, actinomycetes, fungi, algae, and protozoa, five major groups of microorganisms in soils, may have either a favorable or a detrimental influence on plant development. Because the microflora is so intimately associated with the root system, partially covering its surface, any beneficial or toxic substance produced can cause an immediate and profound response. Modifications in the abundance of microorganisms or in the relative proportions of individual groups will in turn affect the plant through the microbiologically catalyzed

reactions. In some examples, the production of CO<sub>2</sub> in the rhizosphere and the formation of organic and inorganic acids aid in the solubilization of inorganic plant nutrients. At the same time, the vast microscopic community demands a variety of anions and cations for its own development, and immobilization of nitrogens or phosphorus may assume prominence. Aerobic bacteria remove O<sub>2</sub> from the environment and add CO<sub>2</sub>, and either the lowering of the O<sub>2</sub> or increasing the CO<sub>2</sub> tention may reduce root elongation and development or diminish the rate of nutrient and water uptake. The rhizosphere microflora may, however, favor plant development by producing growth-stimulating substances, contributing to the formation of a stable soil structure, releasing elements in organic forms through the mineralization of organic complexes, and by entering into symbiotic root associations. Proposing a mechanism for a beneficial or detrimental relationship is far simpler than providing the experimental results, but the evidence for several specific associations has been obtained.

As the above mentioned, this experiment carried out to examine the effects of microbial fertilizers on young radish growth and chemical components of the plant.

## II . Literature Review

The most direct approach to the establishment of the significance of rhizosphere microorganisms is by comparing plant growth in sterile and nonsterile environments. Typically, the rate of development was more rapid in sterile soil receiving an inoculum of organisms than in the uninoculated, sterile controls.<sup>6)</sup>

Phosphorus availability was influenced by the microscopic rhizosphere inhabitants, and because crops required appreciable quantities, changes in the assimilable phosphate concentration were of considerable consequence. A high percentage of rhizosphere and rhizoplane bacteria was able to degrade organic phosphorus substrates, and the total numbers of these heterotrophs(nonphotosynthetic organisms) were similarly increased in the vicinity of actively metabolizing roots.<sup>16)</sup>

Another phosphorus transformation of agronomic significance was the solubilization of insoluble phosphate-containing compounds. Bacteria associated with the root system might be of assistance in rendering available several substances that were poorly soluble. A comparison of the yields of plants grown in sterile and in nonsterile environments containing insoluble phosphate sources revealed that the response to the

chemicals was greater where microorganisms were active.<sup>19)</sup>

Even the absorption of soluble inorganic phosphate might be affected by heterotrophs. More phosphate was taken up by roots colonized by microorganisms than those maintained aseptically. The greater assimilation by plants supporting bacteria on their belowground portions was reflected not only by more phosphorus in the roots but also in the aboveground portions.<sup>5)</sup> The assimilation of manganese, iron, zinc, and potassium might similarly be stimulated by microorganisms.<sup>1,43)</sup>

Development of an active rhizosphere community has a variety of indirect and direct effects on plant biomass production. A wide variety of rhizosphere microorganisms have been demonstrated to produce plant growth hormones, including indole acetic acid (IAA), gibberellins, and cytokinins.<sup>27,44)</sup>

Nitrogen fixation by free-living root-associated bacteria has been well documented. The taxonomic range of plants involved in such associations was wide, and includes wheat (*Triticum aestivum*), rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*), and various forage grasses.<sup>26)</sup> A variety of N<sub>2</sub>-fixing bacteria have been isolated from the roots and rhizospheres of the aforementioned crops; most commonly these isolates have been members of the genera *Azospirillum*, *Azotobacter*, *Erwinia*, *Klebsiella*, and *Bacillus*.<sup>33)</sup>

*Bacillus polymyxa* was a Gram-positive endosporeforming rod which was capable of atmospheric dinitrogen reduction. This species, a commonly-occurring soil bacterium, has been shown to have a positive effect on growth of *Pennisetum* spp,<sup>39)</sup> and common wheat.<sup>21,24,25,34)</sup> However, mechanisms other than N<sub>2</sub> fixation might be responsible for the stimulatory effect of *Bacillus* species on plant yield. For example, the bacterium might produce phosphate-solubilizing compounds that presumably increase phosphate availability,<sup>32)</sup> antibiotics which might suppress pathogenic organisms in the rhizosphere,<sup>7)</sup> or plant growth substances as reported in *Azotobacter*<sup>7,8)</sup> and *Azospirillum*.<sup>44)</sup>

Plant growth-promoting rhizobacteria (PGPR) were considered a valid alternative to chemicals, but unfortunately their effectiveness often lacked consistency under field conditions.<sup>2,9)</sup>

Pierson and Weller<sup>31)</sup> showed that some mixtures of *fluorescent pseudomonads* were more effective in suppressing take-all of wheat than the single strains applied individually, although their performance was rather erratic. Moreover, Belimov *et al.*<sup>4)</sup> observed that inoculation with bacterial mixtures of nitrogen-fixing and phosphate-

solubilizing bacteria provided a more balanced nutrition for the plants. Multiple strain inocula hence seemed to have great potential in plant growth promotion and biological control. In contrast, Chiarini, L *et al.*<sup>12)</sup> have reported that establishment of large populations of bacterial inoculants on roots did not appear to be essential for plant growth promotion when *Burkholderia cepacia* population was significantly reduced in the presence of *Enterobacter sp.* but not of *Pseudomonas fluorescens*.

Bacterial and fungal isolates from soil or plant roots were able to control plant disease or directly stimulate crop growth.<sup>36)</sup> In China, plant-associated *Bacillus*, mainly *Bacillus cereus* Frankland and Frankland, collectively called yield-increasing bacteria(YIB), have been produced commercially and sold for plant growth and yield promotion since the 1980s.<sup>10,11,23,40)</sup> Average yield increases reported for the use of YIB range from 11% with wheat to 25% with vegetables.<sup>22,23)</sup>

Other strains of *Bacillus*, including *B. subtilis* B908(Ehrenberg) Cohn have been isolated and tested for biological control of plant diseases as well as growth promotion in China.<sup>44)</sup> More recently, *B. subtilis* B908 and *B. cereus* A47-2 and A47-3 gave the most frequent positive growth responses(wheat seedling root weight, shoot weight and shoot length) in the absence of pathogen inoculum.<sup>37)</sup>

Soil microorganisms constitute the basic consumer trophic level of the decomposer subsystem. As such, they controlled the breakdown of organic matter and hence the release of nutrients and their availability for other organisms.<sup>18,38)</sup> Further, microbial activity and biomass dynamics helped to regulate long-term soil properties such as net fluxes and amounts of soil carbon and nutrients.<sup>3,13,15)</sup>

The soil microbial biomass contributed to maintaining long-term agricultural sustainability, since the nutrients released due to microbial activity and mineralization of nutrients immobilized in the biomass itself were determinants of temporal patterns of nutrient availability, soil nutrient status and ultimately the net productivity of the agroecosystem.<sup>13,35)</sup>

More indirect, but clearly beneficial, results of stimulation of soil community by root invasion were the contribution of rhizosphere microbial communities to development of a stable soil structure conducive to plant community development.<sup>41)</sup>

Additionally, plant growth stimulation might result from solubilization of inorganic plant nutrients by producing organic or inorganic acids by rhizosphere organisms. Microbially produced organic acids might solubilize essential minerals simply through acidification of rhizosphere soil or by chelation(e. g., siderophore production) of the metal.<sup>28,29)</sup>

### III. Materials and Methods

Seeds(Chungdabok) of young radish(*Raphanus sativus* L.) from Hungnong Seed Company were sown at PE house. All plots were fertilized with 10kg of nitrogen per 10a, 15kg of phosphate per 10a, 8kg potassium per 10a and below mentioned six microbial fertilizers on 20 June 1999 prior to sowing at the Dongguk Univ. experiment field, Ilsan, Kyunggi, Korea. This experiment was a completely randomized block design with four replications. In the mid of July 1999, young radish plants and soils were taken from each plot. Fresh weight, the number of leaves, leaf length, and leaf width of the plant materials were measured. Sugar content of plant materials were measured with Saccarimeter(Model 3131, ATAGO, Japan). The young radish plants were then washed with tapwater to remove all adhering soil. Soil samples taken from the interrows of each plot were divided in half. Plant material and half of soil samples were stored at 80°C. Dry weight of plant materials was measured and then plant materials were ground to less than 0.5mm. Chemical characteristics of plant materials and soil samples were bioassayed from GBC Integra XMP. Data for plant growth and plant chemical, soil chemical, and microbial properties were tested for treatment effects by using analysis of variance. When significant differences occurred, the means were separated using the least significant difference(LSD) test at the 5% level.

Microbial fertilizer treatments were as follows :

The MPK contained Palma, black sugar, calcium superphosphate, and potassium chloride mixed. Palma was obtained from Palma Company.

#### 1) MPK+Husk+Palma treatment

This mix contained a MPK : rice bran : Palma(1 : 5 : 1). One thousand and three hundred eighty six kg of the mix per 10a was then applied.

#### 2) Husk+Palma treatment

This mix contained a rice bran : Palma(5 : 1).

One thousand and one hundred eighty eight kg of the mix per 10a was treated.

### 3) MPK+Compost treatment

MPK : compost(1 : 10) was mixed into 500l of water, and 1l supernatant of this mix solution was then applied in 10 days.

### 4) Compost treatment

Nine hundred ninety kg of a completely decomposed plant residue per 10a was applied.

### 5) BLCS cattle dropping treatment

In the feeds of Holstein milking cow, were 1g BIO Livestock Clean System(BLCS) per day added for the first week from the beginning ; 2g of BLCS per day for the next week ; 5g of BLCS per day for the last week. After the excreta was dried to be 60% moisture content under PE house, 990kg of the completely fermented per 10a was applied.

### 6) Tomi treatment

VS<sub>34</sub>, a microbial fertilizer, which was introduced from Japan is called Tomi.

One hundred ninety eight kg of Tomi and 990kg of rice bran per 10a were applied.

## IV. Results and Discussion

The microorganic properties of microbial fertilizers were analyzed(Table 1). Tomi contained the highest amounts of total bacteria and actinomycetes as  $292 \times 10^6$ (cfu/g) and  $1000.0 \times 10^4$ (cfu/g), respectively.

BLCS cattle dropping contained the highest amounts of both *Bacillus* and yeast as  $215 \times 10^5$ (cfu/g) and  $29.0 \times 10^5$ (cfu/g).

MPK also included *Pseudomonas* and fungi as  $2600 \times 10^4$ (cfu/g) and  $12700 \times 10^3$ (cfu/g) the highest among the microbial fertilizers.

Tomi showed high amounts of all characteristics compared with other microbial fertilizers, while Palma showed lower amounts of such characteristics.

BLCS cattle dropping hardly had *Pseudomonas*.

Table 1. The microorganic populations of microbial fertilizers. (Unit : cfu/g)

Characteristics	Total bacteria ( $\times 10^6$ )	<i>Bacillus</i> ( $\times 10^5$ )	<i>Pseudo-</i> <i>monas</i> ( $\times 10^4$ )	Actino- mycetes ( $\times 10^4$ )	Fungi ( $\times 10^3$ )	Yeast ( $\times 10^5$ )
Palma	3.7	8	1	3.4	0.6	1.0
Tomi	292.0	100	9	1,000.0	4,860.0	16.0
MPK	5.2	66	2,600	266.0	12,700.0	0.4
BLCS cattle dropping	0.1	215	0	130.0	3,800.0	29.0

The external characteristics of young radish treated with microbial fertilizers were evaluated (Table 2). The yields of young radish were increased in six microbial fertilizer treatments. The fresh matter weight, the number of leaves, and the dry matter weight of young radish in BLCS cattle dropping, the leaf length in MPK+Husk+Palma treatment, the sugar content in Husk+Palma treatment, and the leaf width in Tomi treatment showed the highest amount, respectively.

Husk+Palma treatment and MPK+Husk+Palma treatment showed the highest increase on leaf length as 37.0 cm. Furthermore, Husk+Palma treatment showed highly significant increase on sugar content.

Tomi treatment showed the highest effect on leaf width as 10.7 cm.

The plants in Compost treatment did not show higher effect on any characteristics, although statistically significant.

Results described above showed that the six treatments were highly significant on all growth characteristics. As Brown<sup>6</sup> reported, the rate of development was more rapid in soil receiving an inoculum of organisms than in the uninoculated control.

Table 2. Growth characteristics of young radish in the plots inoculated with microbial fertilizers.

Treatment	Fresh matter weight (g/plant)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Sugar content (%)	Dry matter weight (g/plant)
MPK+Husk+Palma	57.4	8.6	37.0	10.1	5.7	2.9
Husk+Palma	61.9	8.7	37.0	10.3	6.7	3.2
MPK+Compost	61.6	8.5	35.6	10.2	5.4	3.3
Compost	60.1	8.6	36.4	10.0	5.3	3.0
BLCS cattle dropping	64.2	9.1	36.6	10.5	5.4	3.3
Tomi	59.5	8.7	36.2	10.7	5.4	3.2
Control	30.4	7.0	30.5	8.6	5.6	1.6
L.S.D(5%)	10.1	0.7	2.6	0.6	0.3	0.6
L.S.D(1%)	13.9	1.0	3.7	0.9	0.4	0.8
CV(%)		24.3		21.6	10.8	19.2



The effect of microbial fertilizers on chemical components of young radish in microbial fertilizer treatments were examined (Table 3).

Phosphoric acid, potassium, and iron amounts showed significant among treatments.

Phosphoric acid amount of young radish in Tomi treatment was much higher than other treatments. Moreover, iron amount in Tomi treatment showed significant.

Potassium amount of young radish showed the highest in Compost treatment, which is a completely decomposed plant residue, compared with control.

Tomi treatment was assayed to contain not only the highest number of total bacteria but also the highest amounts of phosphoric acid and iron. According to Bowen and Rovira,<sup>5)</sup> the greater assimilation by plants supporting bacteria on their belowground portions was reflected not only by more phosphorus in the roots but also in the aboveground portions.

Table 3. Chemical components of young radish in the plots inoculated with microbial fertilizers.

Treatment	T-N	P <sub>2</sub> O <sub>5</sub>	Ca	K	Mg	Na	Cu	Fe	Mn	Zn
	(%)			(g/kg)						
MPK+Husk+Palma	3.36	1.62	147.6	373.3	29.5	30.2	0.10	7.12	0.27	0.32
Husk+Palma	3.48	1.42	145.0	357.5	29.5	29.3	0.07	7.00	0.28	0.27
MPK+Compost	3.16	1.49	140.8	352.7	28.6	27.0	0.07	6.90	0.23	0.24
Compost	3.24	1.56	139.3	377.1	31.9	29.1	0.09	6.93	0.24	0.28
BLCS cattle dropping	3.08	1.81	138.3	371.0	29.4	31.6	0.09	6.95	0.22	0.24
Tomi	3.13	1.83	152.1	360.9	32.0	29.4	0.14	7.37	0.26	0.30
Control	3.25	1.47	143.1	347.9	32.8	34.0	0.07	6.09	0.27	0.28
L.S.D 5%	NS <sup>2</sup>	0.06	NS	3.2	NS	NS	NS	1.15	NS	NS
L.S.D 1%				4.4						
C. V(%)		15.40		10.7				8.40		

NS<sup>2</sup> No significance

The effect of microbial fertilizers on chemical components of soil in microbial fertilizer treatments were examined (Table 4).

Two components, phosphoric acid and potassium, in soil inoculated by microbial fertilizers showed significant, while the others did not show significant.

Phosphoric acid and potassium amounts in Tomi treatment were increased and potassium amount in Husk+Palma treatment showed the highest among such treatments.

Phosphoric acid amounts in MPK+Husk+Palma, Husk+Palma, and Compost

treatments were lower than control, even though they were significant.

In contrast, the amount of nitrate nitrogen except for Husk+Palma treatment showed considerable differences when compared with control, even though not statistically significant.

Table 4. Chemical components of soil inoculated with microbial fertilizers.

Treatment	pH	NO <sub>3</sub> -N	OM	P <sub>2</sub> O <sub>5</sub>	K	Ca	Mg	Na	EC
	1:5	g/kg	%				g/kg		dS/m
MPK+Husk+Palma	6.06	17.94	1.52	750.9	0.61	5.60	2.02	0.36	2.21
Husk+Palma	6.24	9.94	1.27	715.5	0.70	5.08	1.70	0.24	0.90
MPK+Compost	6.24	22.11	1.83	917.7	0.64	6.75	2.19	0.33	1.73
Compost	6.43	25.83	2.01	739.6	0.49	6.93	2.40	0.29	1.13
BLCS cattle dropping	6.5	30.28	1.75	924.9	0.67	7.63	3.09	0.57	3.71
Tomi	5.97	29.28	1.42	1054.0	0.68	6.04	2.48	0.52	2.54
Control	6.08	6.72	1.51	794.6	0.56	6.36	2.42	0.36	2.73
L.S.D(5%)	NS <sup>2</sup>	NS	NS	154.34	0.12	NS	NS	NS	NS
CV(%)				25.90	29.40				

NS<sup>2</sup> No significance

Table 5 showed the microbial properties of soil inoculated with six treatments. Total bacteria, *Bacillus*, actinomycetes, and fungi in Tomi treatment were increased when compared with control. Of such properties, total bacteria showed the highest number, while the number of *Bacillus* showed the highest in MPK+Husk+Palma treatment.

In the Table 5 and Table 1, the numbers of total bacteria and *Bacillus* were declined and actinomycetes and fungi were increased in Tomi treatment.

In the Table 5 and Table 4, it appeared that the numbers of total bacteria containing *Bacillus* and *Pseudomonas* and actinomycetes in Tomi treatment generally induced increase of phosphoric acid.

In the Table 5 and Table 2, it also considered that the number of total bacteria had an effect on leaf length and sugar content(i.e. MPK+Husk+Palma and Husk+Palma) and that of actinomycetes also had an effect on plant growth, even though that was not directly demonstrated.

Table 5. The microorganic populations of soil inoculated with microbial fertilizers.

(Unit : cfu/g)

Treatment	Total bacteria ( $\times 10^6$ )	<i>Bacillus</i> ( $\times 10^5$ )	<i>Pseudo- monas</i> ( $\times 10^4$ )	Actino- mycetes ( $\times 10^5$ )	Fungi ( $\times 10^4$ )	Yeast ( $\times 10^4$ )
MPK+Husk+Palma	112.6	100.6	0.6	16.0	354.5	3.23
Husk+Palma	128.4	91.9	7.3	9.9	585.0	3.43
MPK+Compost	12.9	10.3	12.5	2.0	27.0	7.90
Compost	8.1	13.0	9.3	1.6	10.6	7.23
BLCS cattle dropping	22.6	7.1	11.0	13.1	20.1	7.67
Tomi	143.8	89.7	5.3	37.8	1,228.1	9.33
Control	23.6	13.0	19.0	6.7	12.6	6.23
L.S.D(5%)	83.4	61.1	NS <sup>z</sup>	11.1	815.0	NS
L.S.D(1%)	116.9	85.6		15.5		

NS<sup>z</sup> No significance

## V. Abstract

This study was conducted to investigate the effects of microbial fertilizers on the yields of young radish(*Raphanus sativus* L.), chemical components of plant and soil, and the microbial floras. Six microbial fertilizers, MPK+Husk+Palma, Husk+Palma, MPK+Compost, Compost, BIO Livestock Clean System(BLCS) cattle dropping, and Tomi were used.

The yields of young radish were increased in six microbial fertilizer treatments. The fresh matter weight, the number of leaves, and the dry matter weight of young radish in BLCS cattle dropping treatment, the leaf length in MPK+Husk+Palma treatment, the sugar content in Husk+Palma treatment, and the leaf width in Tomi treatment showed the highest amount, respectively. The effects of microbial fertilizers on chemical characteristics of young radish and soil were examined. Phosphoric acid amount of young radish in Tomi treatment was much higher than other treatments. Potassium amount of young radish showed high significance in all microbial fertilizer treatments compared with control, and showed the highest in Compost treatment. Two components, phosphoric acid and potassium, in soil inoculated by microbial fertilizers showed significant. Phosphoric acid in the Tomi treatment and potassium in

Husk+Palma treatment were increased. The microorganic populations in soil inoculated with microbial fertilizers were examined. While the number of *Bacillus* in soil was increased in MPK+Husk+Palma treatment, the numbers of total bacteria, actinomycetes, and fungi were increased in Tomi treatment.

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