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# SOIL MICROORGANISMS FOR SUSTAINABLE AGRICULTURE

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## ABSTRACT

In order to consider the role of soil microorganisms for the sustainable agriculture, firstly we should recognized that our global environment has been kept in order mainly by the microbial activities inhabiting in the ecosystems as a decomposer, especially maintaining the balance in natural recycling of biological elements such as carbon and nitrogen. Secondly, various kinds of useful microorganisms should be utilized as an innovative technology, especially, rhizobial and mycorrhizal inoculants. But there are still many problems to be solved how to establish the inoculants in the complex soil ecosystems. A more understanding of ecological studies of inoculants in soils, as well as, plant and microbial genetics study are needed to be done. The inoculants of useful microorganisms should be required for registration and increase the standards in use for the sustainable agricultural production. Thirdly, our efforts should be done in promoting the land application of organic waste materials to maintain the soil fertility, partly for promoting the activities of soil microorganisms in soils. However, there seems to be still a more fundamental and applied studies necessary for activations of the indigenous beneficial soil microorganisms.

## INTRODUCTION

The recent development of scientific knowledge revealed that the pedosphere (zone of soil) which covers few meters of thin earth surface is the most important site for our human beings, not only for food production but also for the conservation of the earth environment. The pedosphere is playing a vital role for the geobiochemical cycling of the biological elements essential for the livings such as carbon, nitrogen, phosphorus, sulfur and so on. In fact, the microorganisms living in soils are the key factor in the nutrient recycling in the soil environment : plant production in the natural ecosystem or crop production in the traditional and modern agricultural practice are mostly dependent on the microbiological activities. The microorganisms in soils, the useful microorganisms in particular, have been expected to be utilized as an innovative technology for the sustainable agriculture in the next decade,





21st century.

## SOIL AS THE TREASURE-HOUSE OF MICROORGANISMS

Microorganisms are the first inhabitant in this earth as the livings since few billions years ago. In the long process of evolution of the living things into the mankind the microorganisms should have diversified, increased and distributed in the pedosphere of the earth. A human being who discovered the microbes first time in the 17th century was Antonie van Leeuwenhoek (1632-1723). A number of microorganisms have been isolated and utilized in the fields of food, medicine, industry, agriculture and others during the 19 to 20th century. More attention has been made recently in applying the microbiology to the field of environmental sciences.

It is well known nowadays that soil in a living body and inhabit a number of microorganisms. One gram of soil contains a total microbes at the level of few billions and more than 4,000 different kind of species estimated by the DNA analysis method recently reported by Torsvik *et. al.* (1990).

The term sustainable agriculture shows an analogy to the terms such as organic farming, natural farming, biological farming, ecological farming which have been based principally on the agricultural practice in the natural soil ecosystem. Japanese agriculture in the ancient time when almost no chemical fertilizer and pesticides were used can be compared with the above. However, what we are aiming here this time is a new technology as the sustainable agriculture that was realized after suffering from the environmental deterioration due to the intensive use of fossil energy resources.

It would not be too much exaggerate to state that microorganisms in soils are taking the major role for the sustainability in the natural soil ecosystems. Soil microorganisms as the decomposer in the ecosystem providing plant nutrients released from the organic matter decomposition and the various kinds of biochemical reactions which contribute nutrient availability as well as the environmental cleansing. If these microbial activities would be inhibited or too much promoted the sustainability in the natural soil ecosystem might be hindered.

## USEFUL SOIL MICROORGANISMS

A plentiful useful microorganisms inhabit in soil and many of them have been isolated and utilized by human beings. *Penicillium* for the production of penicillin and *Streptomyces* for the production of streptomycin are the good examples. In the field of agriculture *Rhizobium* is the only microorganisms that has been utilized widely among farmers for the production of various leguminous crops. However, from the view point of agricultural practice depending on the more biological resources, many research works had been done in the past as the following various aspects by the use of useful soil microorganisms.

* To increase the plant nutrient uptake.
* To promote availability of the slightly soluble plant nutrients.
* To produce the plant-growth promoting substances.
* To stimulate the soil aggregation.
* To control the soil pathogens.
* To control the weed growth.
* To decompose pesticide residues.

Among these possible useful microorganisms the atmospheric nitrogen-fixing microorganisms are the most promising one as an innovative technology for the sustainable agriculture. Various ecological $N_2$-fixing systems are shown in Figure 1. Direct application of the free-living $N_2$-fixing bacteria such as *Cyanobacteria* (blue-green algae) or *Azotobacter* to crop fields have been tried in some countries, but no

**Figure 1** Ecological classification of the $N_2$-fixing systems.

| | | | |
|---|---|---|---|
| Free-living (Non-symbiotic) | Autotrophs | Aerobes | Cyanobacteria |
| | | Anaerobes | Phototrophic bacteria |
| | Heterotrophs | Aerobes | *Azotobacter* |
| | | Facultative-anaerobes | *Klebsiella* |
| | | Anaerobes | *Clostridium* |
| Symbiotic | Root nodules | Legumes | *Rhizobium* |
| | | | *Bradyrhizobium* |
| | | Non-legumes | *Frankia* |
| | Leaf nodules | *Ardisia* | *Klebsiella* |
| | Stem nodules | Sesbania | *Rhizobium* |
| | Lichens | Fungi | *Cyanobacteria* |
| | Water fern | *Azolla* | *Cyanobacteria* |
| Association (Pseudosym-biotic) | Rhizospheres | Plant roots | *Alcaligenes* |
| | Phyllosphere | Plant leaves | *Pseudomonas* |
| | Bacteria | Two bacteria | *Psedomonas* |
| | | | *Arthrobacter* |
| | Intestine | Termite | *Citrobacter* |





remarkable results were observed. In the symbiotic $N_2$-fixing microorganisms the effective inoculation of leguminous crops with rhizobia have shown generally successful results for the crop yield. About 550 genera, 12,000 species of leguminous plant are known at present, but only about 10% of these plant have been examined for nodulation. Many of the nodulated legumes are forest trees called "fertilizer trees" in Japan, and further investigation of the symbiotic relationship is required, particularly for the development of innovative technology in the agroforestry. Similar effort should be made with grain legumes and vegetables which also should be required for more studies from the viewpoint of sustainable agriculture.

Enhancement of known symbiotic relationship to promote more biological $N_2$-fixation or transferring the nodulation system to non-leguminous cereal crops by means of biotechnological method should also be greatly inspired.

There are 13 genera, 342 species of non-leguminous trees about 138 species such as *Alnus, Myrica, Elaeagnus, Casuarina, Coriaria, Ceanothus* are known to make nodules by *Frankia* (*Actinomycetes*). The symbiotic relationship in the non-leguminous tree have not yet well studied. The rest of $N_2$-fixing systems may have a large potential to make use for the innovative technology in sustainable agriculture but very few studies had been carried out from this aspect.

*Rhizobium* inoculation could be one of the most investigated microbial technology being made in the past into improving success of inocula to increase the production of leguminous plants. There efforts include the *Rhizobium* strain selection in nitrogen-fixing ability, competitiveness, stress-tolerant to various environmental factors such as pH, temperature, water stress and so on.

*Rhizobium* present in the soil as free-living or a heterotrophic bacteria and only can start growing in the plant rhizobium. Firstly, a newly inoculated rhizobia should struggle against the several soil condition, physical, chemical and biological factors to survive in the soils. Secondly, the rhizobia have to success in the competition among other indigenous rhizobia for the site of colonization on the tip of root hairs of the host plant. The rhizobia able to penetrate into cortical tissue cell through infection thread, form nodule and change by bacteroids which can fix the atmospheric nitrogen.

Mycorrhiza, a symbiont between plants and fungi, are another important microbiological resource in the natural and agricultural ecosystems. The role of mycorrhizal fungi for plant growth is known to enhance the mineral nutrition such as nitrogen, phosphorus, potassium, sulfur and water, in particular phosphorus. The inocula of some mycorrhizal fungi are commercially available recently in some countries.

## PROMOTION AND ESTABLISHMENT OF USEFUL MICROORGANISMS

Microorganisms have attracted the attention of many scientists as one of the most effective means to adapt as a technology in developing the sustainable agriculture. In case of Japan the National Diet Bill held in 1984 passed the Law of Soil Productivity Improvement in order to prevent any further deterioration of soil and the possible subsequent environmental hazard in the Japanese Islands. Since then all of the governmental institutions, private sectors as well as the farmers have made and being made a greater efforts under the nation-wide campaign for the Soil Improvement Movement (Tsuchi-zukuri undo). Generally, there seems to be two directions in our effort from the view point of soil microbiology *i.e.* organic matter application which promotes useful native microorganisms and artificial inoculations with useful microorganisms.

## PROMOTION OF INDIGENOUS USEFUL MICROORGANISMS

It is well recognized among soil scientists that the long-term application of organic matter in soil can increase the fertility of soil and increase crop growth and yield, although they are not necessary in agreement concerning about the mechanisms involved in this phenomenon. The application of organic matter is considered to increase soil fertility by improving the various physical, chemical and microbiological characteristics of the soils. These beneficial effect has been reported not only with application of the traditional farmyard manure but also with the compost which was made by various organic waste materials such as the city garbage, sludge, bark, animal dung and so on.

As far as the microbiological advantages are concerned the land application of organic materials appears to contribute to the followings : build-up microbial population and biomass carbon, nitrogen, phosphorus, and sulfur, then increase of microbial activities in the nutrient transformations. Increased biomass nitrogen in soil added with various composting materials is shown in Table 1 as an example. The biomass nitrogen is now considered as a direct nitrogen source following by mineralized nitrogen for the crop uptake. The increase of biomass nitrogen due to the organic matter application would promote microbial activities of the successive nitrogen recycling such as the nitrogen fixation and the nitrification.

## USEFUL MICROORGANISMS AS INOCULANTS

The microbial soil-amendment materials are classified in Japan as a group of soil-amendment materials which is not applicable for the Fertilizer Control Law. At the time when the Soil Productivity Improvement Law was established by the Act of Congress in 1984 the microbial soil-amendment materials (Dojyou Kairyozai) were already in the market having more





**Table 1** Amounts of biomass nitrogen in soils treated with 1% of different kind of composts after 5-weeks incubation at 28°C (μg N $g^{-1}$ dry soil)

| Treatments | Mineralized-N in non-fumigated soil (A) | Mineralized-N in fumigated soil (B) | Flush-N (A-B) | Biomass-N* |
|---|---|---|---|---|
| City refuse compost | 38.8 | 23.0 | 15.9 | 23.4 |
| Sewage sludge compost | 76.6 | 56.9 | 19.7 | 29.0 |
| Chicken manure compost | 86.6 | 66.8 | 20.0 | 29.4 |
| Swine waste compost | 65.9 | 48.0 | 17.9 | 26.3 |
| Ammonia sulfate | 213.4 | 208.0 | 5.4 | 7.9 |
| Control | 37.0 | 31.5 | 5.4 | 7.9 |

* Biomass-N=0.68xFlush-N (Shen *et. al.*, 1984)

than one hundred kinds. The microbial soil-amendment materials consists of microorganisms or enzymes as the main component which are considered to be effective in promoting plant growth. These materials are generally together with the carrier to adsorb microorganisms or enzymes and other organic and inorganic substances to support microbial growth (Figure 2).

However, there are many inadequate materials which do not indicate manufacturer, raw material, usefulness, direction for use of quality of the materials. The move for recent Soil Improvement Movement and consideration for the sustainable agriculture promoted an increase of the numbers and the varieties of these materials. Probable usefulness of microbial soil-amendment materials are classified into three groups with following objectives, first is to stimulate the organic matter decomposition, second is to lessen the soil-sickness problem, and third is to improve crop growth, yield and quality. The Ministry of Agriculture, Forestry and Fishery (MAFF) started recently to develop the techniques to investigate the effect of these microbial soil-amendment materials as useful microorganisms in soils.

Soils have a complex ecological factors of physical, chemical and biological. An inoculated microorganism newly introduced into soil is generally decline its population very rapidly. How effectively the inocula compete with the indigenous soil microorganisms is the most essential factor for the effectiveness of the inoculants.

**Figure 2** Components of microbiological soil-amendment materials (Hori, 1988)

**Carriers**
Peat
Vermiculite
Zeolite
Diatomaceous earth

**Inorganic**
Ammonium sulfate
Urea
Superphosphate
Potassium chloride

Microorganisms
Aerobic & Anaerobic bacteria
Cellulose, Pectin, Lignin decomposing bacteria
$N_2$-fixing bacteria
Actinomycetes
Fungi
Enzymes

**Organic**
Rice bran
Rice hulls
Saw dust
Animal wastes
Distillery wastes

Others
Vitamins
Plant oil
Synthetic-polymers
Unknown materials

Unfortunately, there is no such detail data available in most of these microbial soil-amendment materials.

As the example, the survival of inoculated *Bradyrhizobium japonicum* in several soils showed rapid decline in number by various abiotic and biotic effects of soil. When the *Bradyrhizobium* inoculants which carries such as peat or yeast extract medium were applied to soybean rhizosphere a consisting survival rate of the inocula was found in rhiosphere soils. The inoculation of Bradyrhizobia around $10^8$ cells to the plant in spot showed very high nodule number and nodule occupancy, whereas the inoculation mixed with soils (600g of soil) showed very few nodule numbers (Table 2). The nodule occupancy in case of soybean fields is reported as 2 to 10% by Hunt *et al.* (1985). Weaver and Frederick (1974) reported that the inoculants of soybean should contain rhizobia cells $10^4$ times more than the number of indigenous rhizobia in the soils in order to obtain 50 percent of the nodule





**Table 2** Changes in nodule numbers and nodule occupancy by inoculum *B. joponicum* A- 1016 str from soybean grown in the andosol.

| Inoculation method | Nodule site | 7 weeks (A) Nodule No. | 7 weeks (B) Nodule occupancy (%) | 11 weeks (A) | 11 weeks (B) | 14 weeks (A) | 14 weeks (B) |
|---|---|---|---|---|---|---|---|
| Peat moss inoculant spot application | Crown site | 25 ± 6 | 58 c | 35 ± 6 | 100 b | 38± 12 | 69 bc |
| | Other site | 1 ± 1 | 0 a | 1 ± 1 | 77 bc | 0 | - |
| | Total | 26 ± 7 | 56 c | 36 ± 7 | 100 b | 38 ± 12 | 69 bc |
| Peat moss inoculant mixture application | Crown site | 0 | 0 a | 1 ± 1 | 100 b | 2 ± 2 | 100 b |
| | Other site | 2± 2 | 85 bc | 6 ± 6 | 95 b | 1 ± 1 | 100 b |
| | Total | 2 ± 2 | 85 bc | 7 ± 7 | 96 bb | 3 ± 3 | 100 b |
| YEM broth inoculant spot application | Crown site | 22 ± 15 | 91 b | 56 ± 6 | 81 bc | 29 ± 21 | 100 b |
| | Other site | 4 ± 4 | 0 a | 0 ± 0 | - | 0 | - |
| | Total | 26 ± 19 | 79 bc | 56 ± 6 | 81 bc | 29 ± 21 | 100 b |
| YEM broth inoculant mixture application | Crown site | 3 ± 2 | 100 b | 6 ± 6 | 89 bc | 0 | - |
| | Other site | 2 ± 2 | 0 a | 20 ± 3 | 100 b | 6 ± 5 | 100 b |
| | Total | 5 ± 4 | 66 bc | 26 ± 9 | 93 b | 5 ± 5 | 100 b |

* Figures follow by the same letter are not significantly different ($p=0.05$).
Each value of nodule No. represents mean ± S. D. of three replicates.
Each value of nodule occupancy is mean of three replicates.

occupancy.

The mycorrhiza is also of great ecological significance as a biofertilizer concerned with mainly phosphorus cycling. Mycorrhizas are formed by association between plant root and fungus, eventually produce a number of spores. These spores have been generally utilized as inoculants in case of mycorrhizal fungal inoculation. Again, the successful production and application of the spores as inoculants present a great challenge because of the complexity of soil ecological factors in the same was as in the case of *Rhizobium* inoculation. The tripartite association (*Rhizobium*, VAM and Host plant) seems to be more effective for plant nutrition than dual association of *Rhizobium* and host plant (Table 3). At present we are trying to find a technology to trace the fate of mycorrhizal fungi inoculated in soils.

## CONCLUSION

From the above discussion it is clearly

**Table 3** Improvement of the symbiotic $N_2$-fixation of peanut by dual inoculation with VAM and *Bradyrhirobium*.

| Treatment | Non-sterilized soil | | | Sterilized soil | | |
|---|---|---|---|---|---|---|
| | Nodule number plant$^{-1}$ | Nodule plant$^{-1}$ weight(mg) plant$^{-1}$ | %Ndfa | Nodule number plant$^{-1}$ | Nodule weight(mg) plant$^{-1}$ | %Ndfa |
| M-R-(Control) | 194 $^{b}$ | 227 $^{b}$ | 63.5 $^{b}$ | 0 | 0 | - |
| M+R- | 202 $^{b}$ | 238 $^{b}$ | 65.0 $^{b}$ | 0 | 0 | - |
| M-R+ | 221 $^{ab}$ | 244 $^{b}$ | 67.8 $^{ab}$ | 81 $^{b}$ | 88 $^{b}$ | 37.8 $^{b}$ |
| M+R+ | 253 $^{a}$ | 279 $^{a}$ | 75.3 $^{a}$ | 182 $^{a}$ | 152 $^{a}$ | 67.7 $^{a}$ |
| LSD (0.05) | 38 | 32 | 7.4 | 69 | 50 | 12.3 |

M-R- (Control), without any inoculation; M+R-, inoculation with VAM-fungi; M-R+, inoculation with *Bradyrhizobium*, M+R+, dual inoculation with VAM and *Bradyrhizobium*.
% Ndfa, percent nitrogen derived from atmosphere.
In a column, means followed by the same letter(s) are not significantly different at 5% level of significance.

revealed that the microorganisms are the key factor in maintaining the soil ecosystem and playing major role for the sustainable agriculture through recycling the bioelements, decomposing organic matter and supply nutrients to the plants. In order to prevent the decline of soil fertility that appeared to be derived by the use of much fossil energy for the modern agricultural production in Japan the land application of organic materials seems to be useful method in promoting various kinds of useful soil microorganisms. However, inoculation of isolated useful microorganisms, so-called microbial soil-amendment materials should be used with careful considerations. The technology to establish artificial inoculants in natural soils should be further investigated. The inoculants should be specified about the contents, how to trace in soil environment and effectiveness to crop yield having statistically significant benefits. The Japanese standard for inoculant, particularly for the rhizobial and mycorrhizal inoculant products should be applied as quickly as possible.

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