CURRENT STATUS AND FUTURE DIRECTION OF COMMERCIAL PRODUCTION AND USE OF BIO-FERTILIZERS IN JAPAN

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ABSTRACT

Currently, the percentage of annual self-sufficiency of soybean and corn seeds is extremely low in Japan. Four million tons of soybean seed and 16 million tons of corn seed are imported from other countries such as the United States and Brazil. A large part of the imported soybean and corn seeds are used as feeds for domestic animals. This caused serious problems concerning a large amount of waste obtained from animal husbandry. Under this circumstance, various kinds of organic nitrogen fertilizers containing wastes are supplied to farmers' fields. Hence, a large part of farmers in Japan do not recognize the importance of using bio-fertilizers. The Japanese government does not authorize most bio-fertilizers with the exception of VA mycorrhiza. However, the VA mycorrhiza is an ordinance-designated soil conditioner in the country. Thirty-five (35) tons of VA mycorrhiza was produced in Japan in 2004. The output of the VA mycorrhiza showed the highest value in 2001, but the production level is currently tending downward. The Tokachi Federation of Agricultural Cooperatives (TFAC; Japanese abbreviation: Tokachi Nokyoren) in Hokkaido is the major producer and distributor of the rhizobium bio-fertilizers in Japan. In TFAC, three (3) kinds of bio-fertilizers are produced and sold presently. "Mamezo" is a normal type of bio-fertilizer for soybeans, azuki beans and phaseolus beans. Rhizobium and Bradyrhizobium are mixed with peat and natural organic matters. The second type of bio-fertilizer is "R-Processing Seeds", wherein leguminous seeds are inoculated with rhizobia. The final type of bio-fertilizer is "Hyper Coating Seeds". In hyper coating seeds, leguminous grass seed are coated by rhizobia within the capsule of calcium carbonate. These bio-fertilizers are being used by about 80% of farmers in Hokkaido. Furthermore, during a field test done in 2000, treatment applied to soybean using Mamezo containing Bradyrhizobium and Azospirillum inoculants showed 35% increase in soybean seed production compared to that of soybean treatment without inoculants.

Key words: bio-fertilizer, *Bradyrhizobium*, mycorrhizae, Japan, soybean seed, corn seed, Mamezo, VA mycorrhiza

INTRODUCTION

Feeding the rapidly growing populations in developing countries is the most important challenge for mankind. Presently, about 800 million people in the world are suffering from chronic malnutrition due to shortage of suitable foods. In the mid 21st century, we will have depleted reserves of oil (in a span of about 40 years) and natural gas (in a span of about 60 years) under the shallow surface of the earth. This will lead to rise in costs of chemical nitrogen fertilizers and agro-chemicals, and eventually to reduction of their production. To solve the risk of decrease in agricultural productions, we should develop alternatives to the use of chemical nitrogen fertilizers and agro-chemicals. Under above circumstances, the most promising way is developing agricultural microorganisms into bio-fertilizers and bio-pesticides which supply plant nutrition and fight plant pathogens. However, effects of the

Table 1. Annual amount of imported soybean and corn seeds and percent of their annual self-sufficiency

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
										x1000ton
Annual amount of imported soybean	4,870	5,057	4,751	4,884	4,829	4,832	5,039	5,137	4,407	4,181
Percent of annual self-sufficiency	3	3	3	4	5	5	5	4	3	—
										x1000ton
Annual amount of imported corn	16,258	16,083	16,245	16,422	15,982	16,276	16,788	17,012	16,248	16,798
Percent of annual self-sufficiency	0	0	0	0	0	0	0	0	0	—

bio-fertilizers and bio-pesticides are sometimes unstable compared with that of chemical materials. To achieve stable effects of such bio-materials and chemical materials on crop productions, we need to further investigate and promote the use of bio-fertilizers and bio-pesticides.

Bio-fertilizer is a substance containing living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey 2003). This definition distinguishes bio-fertilizer from organic fertilizer. The latter contains organic compounds which directly, or by decay, increase soil fertility. Likewise, the term bio-fertilizer should not be used interchangeably with terms such as green manure or manure, intercrop, or organic-supplemented chemical fertilizer. Not all plant growth promoting rhizobacteria (PGPR) can be considered as bio-fertilizers. Bacteria that promote plant growth by control of deleterious organisms are bio-pesticides. Similarly, such bacteria can enhance plant growth by producing phytohormones and are regarded as bio-enhancers, not bio-fertilizer. Interestingly, some PGPR can promote growth by acting as both bio-fertilizer and bio-pesticide or bio-enhancer.

In this paper, we shall introduce the current status and future direction of commercial production and use of bio-fertilizers and bio-pesticides in Japan.

COMMERCIAL PRODUCTION AND USE OF BIO-FERTILIZERS IN JAPAN

Before discussing Japanese bio-fertilizer, we would explain the present condition of Japanese agricultural production. In the case of soybean and corn, percent of annual self-sufficiency and annual amount of imported seeds are shown in Table 1. Four million tons of soybean seeds and 16 million tons of corn seeds are imported from other countries such as the United States, Brazil, and so forth. Large part of the imported soybean and corn seeds are used for feeds of domestic animals. This caused serious problems on a large amount of waste obtained from animal husbandry. For example, in 2004, more than four million tons of domestic animal wastes are utilized as organic manure (Agricultural statistics in Ministry of Agriculture, Forestry, and Fishery Japan, www.maff.go.jp/j/tokei/index.html). This means that more than seventy thousand tons of ammonia (or more than 350 thousand tons of ammonium sulfate) is fertilized into farmers' field every year. Under such situation, various kinds of organic nitrogen fertilizers are supplied to fields, hence, a large number of farmers in Japan cannot deeply consider bio-fertilizer use as important. However, the Ministry of Agriculture, Forestry, and Fishery in Japan established the law promoting organic agriculture in December 2006. This law increased interests among farmers in organic agriculture involving the use of bio-fertilizer.

Vesicular-arbascular mycorrhizae inoculant

The beneficial effects of mycorrhizae on plant growth are well documented. Their most important benefit is the increase in uptake of immobile P from the soil. The predominant mycorrhizal associations that influence root P absorption involve the vesicular-arbascular (VA) mycorrhizae. VA mycorrhizae are the most abundant mycorrhizae that colonize roots of a wide range of plant species living in almost all types of soils. They form extensive hyphal networks in the soil and within root cortical tissues, forming intimate associations with the plasma membrane without penetrating the cells (Kochian 2000).

The Japanese government does not authorize most bio-fertilizers except the VA mycorrhiza. However, the VA mycorrhiza is an ordinance-designated soil conditioner in Japan (Table 2). Thirty five tons of the VA mycorrhiza was produced in Japan in 2004. Several companies supply VA mycorrhiza inoculants with carriers (Fig. 1 and Fig. 2). Inoculants cost around $83 to $100 per 5 kg.

Table 3 shows production of the VA mycorrhiza inoculants and their output in Japan. The output of the VA mycorrhiza showed the highest value in 2001, but its production is currently tending downward.

Bradyrhizobium inoculant

Soybean [*Glycine max* L. (Merrill)] is a major leguminous crop world-wide, and is extensively cultivated in Eastern Asia and South Eastern Asia. The genus *Bradyrhizobium* is a slow growing, gram negative kind of soil bacteria, and is a major symbiont of soybeans. Based on 16S rRNA gene sequences, the genus *Bradyrhizobium* was classified into a clade in the Proteobacteria along with oligotrophic soil, or aquatic bacteria such as *Rhodopseudomonas palustris*, *Rhodoplanes roseus*, *Nitrobacter winogradskyi*, *Blastobacter denitrificans*, and the pathogen *Afipia* spp. (Saito *et al.* 1998; Sawada *et al.* 2003; van Berkum and Eardly, 2002; Willems *et al.* 2001). The genus *Bradyrhizobium* currently consists of *Bradyrhizobium japonicum* (Jordan, 1982), *B. elkanii* (Kuykendall *et al.* 1992), *B. liaoningense* (Xu *et al.* 1995), *B. yuanmingense* (Yao *et al.* 2002), *B. betae* (Rivas *et al.* 2004), and *B. canariense* (Vinuesa *et al.* 2004). Under appropriate environmental conditions, soybean and *Bradyrhizobium* can initiate a symbiotic interaction, resulting in the development of nitrogen-fixing root nodules.

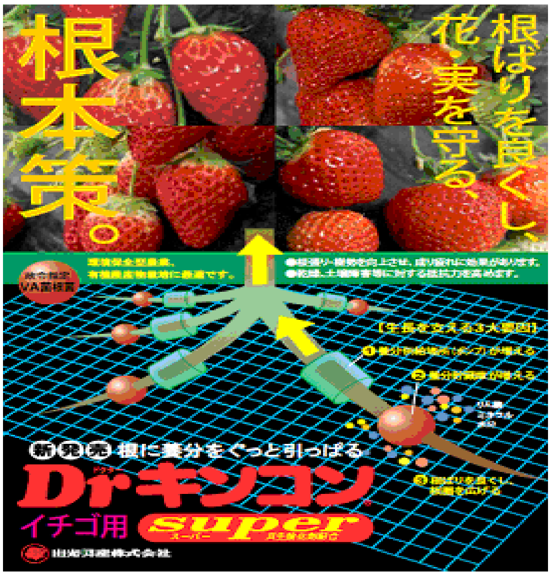
The Genus *Bradyrhizobium* is known to display a large degree of antigenic heterogeneity, and can be categorized into several serological groups, based on differences in somatic antigens. Date and Decker (1965) classified the United States' indigenous *Bradyrhizobium* strains into a total of 17 serological groups and 24 antigens. Thereafter, Keyser *et al.* (1984) reported that serological characterization of 972 isolates of *Bradyrhizobium* were obtained from 65 soybean field locations in 12 states. The most

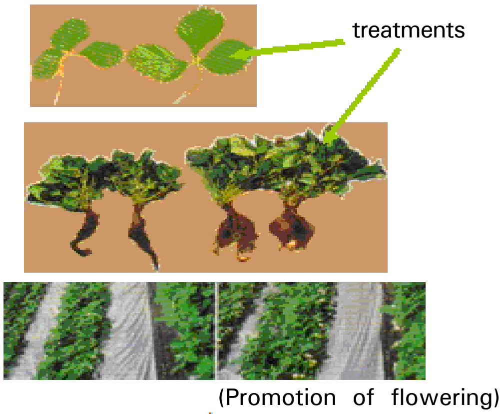
Table 2. Production of ordinance-designated soil conditioners in Japan

(tons)

Materials	1998	1999	2000	2001	2002	2003	2004
Peat	94,765	81,632	75,650	60,661	67,090	61,241	62,213
Bark compost	448,722	451,317	415,139	364,744	352,075	304,909	267,246
Humic substances	26,230	24,248	31,128	33,882	24,787	12,913	13,447
Chacoal	7,197	3,644	7,333	7,343	6,576	7,386	7,263
Diatom soil	885	822	800	805	605	210	533
Zeolite	35,125	46,917	35,266	38,651	32,151	24,145	25,031
Vermiculite	13,501	19,832	19,786	14,572	21,022	18,727	21,709
Perlite	20,160	24,947	28,273	14,204	25,396	10,112	11,630
Bentonite	1,837	1,607	1,385	1,270	1,160	1,627	976
VA mycorrhiza	**25**	**28**	**55**	**56**	**39**	**42**	**35**
Polyethyleneimines	200	208	210	218	220	221	229
Polyvinylalcohols	12	350	281	34	10	39	38
Total	648,659	655,552	615,306	536,440	531,131	441,572	410,350

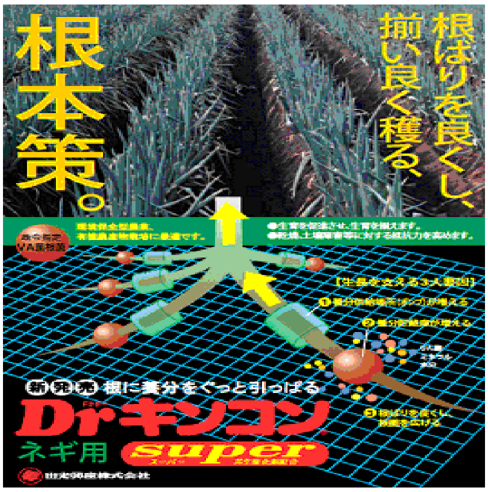
Fig. 1. Dr. Kinkon (Dr. Mycorrhiza) for strawberry.





5kg/pack
Mix inoculants in 500kg culuture media
(Dilution ratio is at 100 times)

Fig. 2. Dr. Kinkon (Dr. Mycorrhiza) for leek.



Figs. 1. and 2. Two kinds of commercial inoculants for strawberry and leek, respectively. In strawberry, the inoculants promote growth and flowering.

Table 3. Production of VA mycorrhiza inoculants and their output in Japan

Materials	1998	1999	2000	2001	2002	2003	2004
Production of VA mycorrhiza inoculants (ton)	25	28	55	56	39	42	35
Output (million$)	2.5	2.8	5.5	5.6	3.9	4.2	3.5

predominant serogroup was 31 (21.5%), followed by 123 (13.6%), shown in Table 4. Sawada *et al.* (1989) used antisera prepared against USDA *Bradyrhizobium* strains to determine the serological properties of Japanese isolates shown in Table 5. In the case of Japanese soybean fields, the most predominant serogroup was 110 (27.1%), while frequency of serogroup 110 in the US soybean fields shown in Table 4 was 1.5%. We know that *B. japonicum* USDA 110 is applied to soybean fields world-wide as an effective inoculant. These results show that effective isolates categorized into serogroup 110 are widely distributed in Japanese soybean fields. Under this circumstance, farmers from the middle and Souther part of Japan generally do not apply *Bradyrhizobium* inoculants to their soybean fields. However, in the Northern part of Japan (Hokkaido area), many farmers apply *Bradyrhizobium* inoculants to soybean.

The Tokachi Federation of Agricultural Cooperatives (TFAC: Japanese abbreviation: Tokachi Nokyoren) in Hokkaido is a major producer and distributor of *rhizobium* bio-fertilizers in Japan. The TFAC is located in Tokachi-Obihiro area in Hokkaido, the most northern prefecture in Japan, and it started the *rhizobium* bio-fertilizer business in 1953. At present, TFAC produces and sells three kinds of biofertilizers. Mamezo is a normal type of bio-fertilizer for soybeans, azuki beans and phaseolus beans shown in Fig. 3. *Rhizobium* and *Bradyrhizobium* are mixed with peat and natural organic matters. The second type of bio-fertilizer is the R-Processing Seeds, which are leguminous seeds inoculated with rhizobia. The final type of bio-fertilizer is the Hyper Coating Seeds shown in Fig. 4. In the hyper coating seeds, leguminous grass seeds are coated with rhizobia within the capsule of calcium carbonate. These bio-fertilizers are

Table 4. Serogroup frequencies of *Bradyrhizobium* isolates in soybean fields in the United States

Serogroup	No. of isolaes	% of total	Species
31	209	21.5	*B. elkanii*
123	132	13.6	*B. japonicum*
76	99	10.2	*B. elkanii*
46	84	8.6	*B. elkanii*
6	74	7.6	*B. japonicum*
135	59	6.1	*B. japonicum*
94	49	5	*B. elkanii*
122	41	4.2	*B. japonicum*
110	15	1.5	*B. japonicum*
unknown	95	9.8	unknown

Keyser *et al.* (1984)

Table 5. Serogroup frequencies of *Bradyrhizobium* isolates in soybean fields in Japan

Serogroup	No. of isolaes	% of total	Species
110	23	27.1	*B. japonicum*
unknown	20	23.5	unknown
125-127	8	9.4	*B. japonicum*
122-129-A1017	7	*8.2*	*B. japonicum*
144	5	5.9	B. elkanii
6	4	4.7	B. japonicum
5033	4	4.7	unknown
4	3	3.5	B. japonicum
62	3	3.5	*B. japonicum*
46	3	3.5	*B. elkanii*
123-129	2	2.4	*B. japonicum*
76	2	2.4	*B.elkanii*
39	1	1.2	*B. elkanii*

Sawada *et al.* (1989)

Fig. 3. Mamezou is a normal type of inoculant of Rhizobia for soybeans, azuki beans and phaseolus beans.

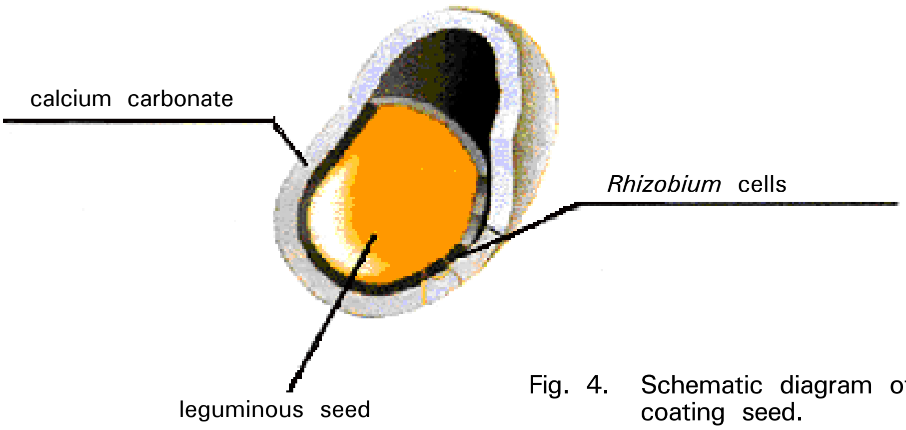


Fig. 4. Schematic diagram of a hyper coating seed.

being used by about 80% of farmers in Hokkaido. TFAC shows that these bio-fertilizers have the capacity to enhance legume growth, promote high seed yield and protein-rich seeds. In soybean field cultivation using the crop rotation system, 1.2 times of nodulation is possible and about 4% increase in soybean yield is substantiated by inoculation, compared to non-inoculated case, in average. The TFAC facility, with two production lines for producing the concentrated *Rhizobium* inoculant paste, was completed in 1990. Microorganisms are cultured and propagated with sucrose and concentrated by a centrifuge, and then frozen for storage. The frozen culture is thawed and mixed with sterilized peat (carrier) to produce bio-fertilizers. TFAC uses peat imported from Canada as bio-fertilizer carrier and acetylene reduction method is adopted to measure the N2 fixation efficiency. The market price of Mamezo is about US$5/40g pack for 10, while the cost of microorganism itself is about US$0.6. Furthermore, mix–inoculation treatment with *Bradyrhizobium* and *Azospirillum* to soybean showed 35% increase in soybean seed production compared to soybean seed treatment without inoculants in a field test done in 2000 (Table 6) (http://www.agri.pref.hokkaido.jp/center/kenkyuseika/gaiyosho/h13gaiyo/2001304.htm)

If the yield increase is consistent in farmers' fields, the inoculants containing rhizobium and *Azospirillum* promise great future for bio-fertilizers used in soybean cultivation in the Hokkaido region.

Table 6. Yields of soybean seeds under different treatments of inoculation systems
(Non-inoculation, *Bradyrhizobium*, *Bradyrhizobium* + *Azospirillum*)

Treatment		Middle stage of growth			Harvest					
Methods of inoculation	Treatments	Soot length (cm)	No. of branches (/plant)	No. of nodules (/plant)	Total weight (kg/10a)	Weight of shoots (kg/10a)	Weight of pods (kg/10a)	No. of pods (/plant)	Weight of matured pods (kg/10a)	Index
Apply to soil	non	37.8	5.5	1.78	2207	1023	1184	63.0	1154	103
	Bradyrhizobium	41.6	6.1	2.84	2752	1428	1324	66.0	1273	114
	Brady + Azospirillum	50.2	7.4	2.89	3305	1775	1530	83.1	1510	135
Apply to seeds	Non	37.9	4.7	1.91	1893	758	1135	58.1	1121	100
	Bradyrhizobium	37.7	5.3	2.01	2202	1043	1159	56.8	1138	102
	Brady + Azospirillum	41.2	5.4	2.32	2427	1172	1255	63.6	1234	110

Price of domestic soybean seeds: Cooked beans 35$ - 103$/60kg; Nattou beans 35$ – 57$/60kg; Tofu beans 31$ – 51$/60kg.
Every plot was applied with nitrogen fertilizer at 2.6kgN/10a.
(Hokkaido Central Agricultural Experiment Station 2000)

Table 7. Soybean seed production in Japan (2003)

	Area planted to soybean (ha)	Seed yield (kg/10a)	Soybean harvest (ton)
The whole country	149900	180	270200
Hokkaido area	20000	208	41500
Touhoku area	38300	134	51400
Hokuriku area	19000	175	33200
Kanntou+Touzann area	18600	191	35500
Toukai area	9650	167	16100
Kinnki area	8210	164	13500
Chugoku area	7840	145	11400
Shikoku area	2040	144	2940
Kyuusyuu area	26300	246	64700

Cooked bean: 83$/60kg (1.4$/kg)

FUTURE DIRECTION OF BIO-FERTILIZER IN JAPAN: A CASE STUDY OF THE BENEFITS OF BIO-FERTILIZER IN HOKKAIDO AREA

The soybean seeds production in Japan in 2003 are shown in Table 7. In this case study, we supposed that mix-inoculants are directly applied to soil as shown in Table 6. The cost of inoculants is $83/ha and 1 kg of soybean seeds gives $1.4. Based on the data in Tables 6 and 7, soybean without inoculation yields about 2,080 kg of seeds/ha. In this case, the income generated from 1 ha of soybean field is about $2,800. Soybean cultivation with *Bradyrhizobium* inoculants showed 14% increase of seed production in Table 6. In this case, the income obtained from 1 ha of soybean field is about $3,300. The table further showed that soybean cultivation with both of *Bradyrhizobium* and *Azorhizobium* caused a 35% increase in seed production. In this case, an income obtained from 1 ha of soybean field is about $3,900. This economical calculation on inoculants usages is very promising to Japanese farmers in Hokkaido area.

The average income from soybean seeds production and the average expenditure in fertilizer per farmer amount to around $17,000 per year in Hokkaido. If seed yields with *Bradyrhizobium* and *Azospirillum* inoculants become 35% higher than those without inoculation, farmers' income increases for around $6,000. If this evaluation is reasonable, farmers will be interested in using bio-fertilizers. However, in the remaining regions in Japan, the farmers' income from soybean seeds production is too small. Therefore, to promote bio-fertilizer usage for increased soybean seed production level in the remaining areas in Japan, we need to develop a new technology adapting to the different agricultural systems in Hokkaido.

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