CURRENT STATUS OF BIO-FERTILIZERS DEVELOPMENT, FARMERS' ACCEPTANCE AND UTILIZATION, AND FUTURE PERSPECTIVE IN TAIWAN

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ABSTRACT

Taiwan is a subtropical-tropical island characterized by high temperature and heavy rainfall. Intensive agricultural practices have served as a strong foundation for Taiwan's commercial and industrial economic miracle. In recent years, chemical pesticides and fertilizers were extensively applied to obtain higher crop yield. Overusing agrochemicals led to several agricultural problems such as poor cropping system. The excessive application of chemical nitrogen fertilizer accelerated soil acidification and threatened air and groundwater contamination at the same time. Using organic fertilizers and bio-fertilizers offer a safe option for reducing the agrochemical inputs. Over the years, bio-fertilizers have been developed in several laboratories in Taiwan. Beneficial microbes such as rhizobium, associative and free living nitrogen-fixing bacteria, phosphate-solubilizing bacteria (PSBs), arbuscular-mycorrhiza (AM), fungi, organic materials, and decomposing microorganisms are considered as bio-fertilizers. These are continuously isolated from various ecosystems, and their performances and field conditions are assessed in the laboratories. The extensive research program on beneficial bacteria and fungi has resulted in the development of a wide range of bio-fertilizers, which satisfied the nutrient requirements of crops and increased the crop yield as well. Many experiments in greenhouses and in field conditions have revealed that different crops responded positively to microbial inoculations. In particular, successful rhizobial inoculants were applied to leguminous plants and AM fungi for muskmelons in order to increase the yield and quality. Multifunctional bio-fertilizers were developed to reduce about 1/3-1/2 of chemical fertilizer application. In the future, enhancement and maintenance of soil fertility through microorganisms will be a very significant concern. Long-term conservation of the soil's health is the key benefit of bio-fertilizers, which is vital in sustainable agriculture.

Key words: bio-fertilizers, status, acceptance, perspective, sustainable agriculture, inorganic fertilizers, inoculants

INTRODUCTION

Taiwan experiences warm and heavy rain weather, averaging around 20°C (November to April) and above 30°C (June to November). Rainfall amounts to over 3,000 mm in central region, 1,500-3,000 mm in the east coast, and 1,500-2,000 mm in western region adjacent to mountains with frequent typhoons during summer and autumn affecting the agriculture industry. The dynamic variations of soil chemical and biological properties are extreme, so lots of chemical fertilizers, pesticides and herbicides are needed to be applied to provide nutrients for the growth of crops and to control the populations of pathogens, insects and weeds. Uncontrolled application of chemical fertilizer is a common scenario. The estimated consumption of chemical fertilizer in Taiwan was about 1,140 thousand tons for every 844 thousand hectares.

Thus, reducing the quantities of agrochemicals applied to soils without decreasing crop production is indeed a serious problem in Taiwan. It is well a known fact that the rhizosphere of crop provides a unique niche for the association of symbiotic and non-symbiotic microorganisms (Young 1994). N_2-fixing and phosphate-solubilizing bacteria have been well characterized from various soil types and aquatic environments (Seshadri *et al.*, 2000; Garg *et al.* 2001; Berman-Frank *et al.*, 2001; Young *et al.* 1982; Young and Cheng, 1998; Young *et al.* 2003 a,b) and many of these isolated bacteria were successful in improving crop yields (Subba Rao 1982; Young *et al.* 1988a; Young 1994). Hence, several beneficial microorganisms can be effectively used as a chemical fertilizer alternative to minimize the application of inorganic fertilizers and increase the nutrient absorption efficiencies of crops. This paper briefly reviews the status of bio-fertilizers in Taiwan, which have been developed in some laboratories in the country, as well as the extensive researches that have been carried out over the years.

CURRENT STATUS OF BIO-FERTILIZERS DEVELOPMENT

The main and direct purposes of applying bio-fertilizers to soil are: to provide nutrient sources and good soil conditions for the growths of crops when used as a live body;, to partially substitute and enhance the function of chemical fertilizer and then subdue the application quantities of fertilizers and still maintain the same crop yields and the capital used for making bio-fertilizers is cheaper than that of chemical fertilizers and to lessen the negative effect aroused from applying chemical fertilizers to soil. On the other hand, the indirect purposes of using bio-fertilizers to soil are: to enhance the growth of root system to increase the water and nutrient absorption abilities of crops, extend the life of root, neutralize and degrade harmful materials accumulated in soil, promote survival efficiency of seedling after transplanting and get shorter time for the flower to come out.

The current researches and development of bio-fertilizers varies in different aspects such as follows:

1. Rhizobial Inoculants

In Taiwan, research work in the selection of efficient rhizobial strains for inoculation started in 1958. Collection, isolation and subsequent selection of effective rhizobial strains and its uses in agriculture have yielded fruitful results. Significant variations were observed among rhizobial strains (Young and Chao 1983). Wu (1958) selected a number of pure rhizobial strains from lupin, alfalfa, peanut, crotalaria and soybean and conducted a wide range of field experiments to select the effective inoculants. Yields were significantly increased when lupin, alfalfa, peanut and soybean were inoculated with selected rhizobial strains compared to those with non-inoculated plants.

After the 1980s, slow and fast-growing soybean rhizobial strains were isolated and selected from Taiwan soils for inoculation (Young *et al.* 1982; Young and Chao 1983). Several effective isolates were deposited in the Culture Collection and Research Center (CCRC) of the Food Industry Research and Development Institute in Taiwan (CCRC 1991).

Few field experiments were conducted to determine the effects of single and mixed inoculations with rhizobium and Arbuscular-Mycorrhiza (AM) in six different tropical soils found in Taiwan (Young *et al.* 1988b). The results indicated that inoculation with rhizobial strains alone increased N_2 fixation and soybean yield in three out of six fields. Inoculations with rhizobial strain singly, or in combination with AM, without any N_2 fertilizer applications, significantly increased soybean yield from 5% to 134% in the field experiments. The results from the other experimental site also showed that a mixed inoculant of rhizobium and AM can be an efficient biological fertilizer that maximizes soybean yields. The combined effect of the mixed inoculant was a striking finding in the field of bio-fertilization. The AM might have provided the essential P for the growth of soybean plants.

2. The effect of inoculating associative and free living nitrogen-fixing bacteria on the growth of crops

Corn seed blended with peat moss was mixed with *Azospirillum* sp. suspension (3.6×10^8 cfu ml^{-1}) at a ratio of 1:5 (w:w) and sowed in the field. The experimental result showed the growth of corn could be significantly promoted and that the flowering time of the corn became one week earlier than that of corn seed without *Azospirillum* sp. suspension (Chien and Chang, 2005). The fresh weights of melon and papaya seedling inoculated with *Azotobacter* sp. suspension (10^7 cfu.ml^{-1}) once a week were 68% and 103% respectively, heavier than those of seedlings without inoculating suspension (Chien and Chang, 2004).

3. Phosphate-solubilizing microbial inoculants

Phosphate-solubilizing bacteria (PSBs) were isolated from various soils in Taiwan. Aliquots of soil diluted in sterile water (1:10, w:v) were plated on calcium phosphate medium (modified from Subba Rao, 1982) for the isolation of Phosphate-solubilizing bacteria.

The basic research on phosphate-solubilizing bio-fertilizers was successfully established during 1990s in Taiwan (Young, 1990; Chang and Young, 1992a,b; Young *et al.*, 1998a,b; Young and Chen, 1999; Chang and Young, 1999; Young *et al.,* 2000; Liou and Young, 2002; Young, *et al*., 2003). Crop plants such as peanut, various horticultural plants and vegetables were successfully inoculated with PSBs to obtain higher yields. Several field experiments concluded that PSBs do not only improved the growth and quality of crops but also drastically reduced the usage (by 1/3-1/2) of chemical or organic fertilizers.

Tomato and muskmelon seeds were immersed in PSB suspensions; diluted to 10^{-3} with Hoagland's solution for 1 minute and sowed in pot containing vermiculite to carry out the experiment. Experimental results showed that the suspensions of PSBs significantly promoted the height and fresh weight of tomato and muskmelon seedlings, respectively. The propagating stem length and growth tendency of muskmelon inoculated with TARIB108 were larger than those of control treatment after field experiment was carried out for six weeks. The average fruit weight and volume of muskmelon inoculated with TARIB108 suspension was 50-g heavier and 24 cm^3 larger than that of non-inoculated treatment. The sweet index (Brix) of muskmelon inoculated PSB TARIB108 was 2 degrees higher than that of non-inoculated treatment. The propagating stem length of watermelon inoculated with PSB suspension was 60 cm longer than that of non-inoculated watermelon (Chien and Chang, 2004).

The suspensions (5×10^8 cfu.ml^{-1}) of *Klebsiella* sp., *Bacillus* sp. (PSBs) and *Azospirillum* sp. (associative nitrogen-fixing bacterium) were mixed at equal volume and then added to spent mushroom sawdust compost at a ratio of 1:100 to make corn crop have significantly higher and heavier shoot than those of control treatment in a pot cultural experiment (Chien, 2005).

4. AM Inoculants

The major AM fungi used for inoculation were *Glomus* spp. isolated from tropical soil of Taiwan (Young, 1986). Chlamydospores were borne terminally on single undifferentiated hyphae in soil. The mature spores were separated from the attached hyphae by a septum. The AM fungal inoculant was placed in pots containing sterilized mineral attapulgite $[(Mg.Al)_5Si_8O_{22}(OH)_4.4H_2O]$ with *Zea mays* as the host plant. The AM inoculant used in pot experiments contained approximately 50 spores per gram of soil together with infected roots (Young *et al.* 1988).

Young *et al.* (1986) used two species of AM in a pot experiment to observe the effect of inoculation of AM fungi in the yield and mineral P utilization in soybean. The results showed that the AM fungi inoculation increased soybean yields over the uninoculated treatments to certain extent depending on the soil type. Moreover, the P uptake by soybean was significantly improved in the inoculated treatments. In a similar experiment, rhizosphere soil was used to assess the difference in P uptake by the soybean plants. Soybean in non-inoculated treatments took up minimum Al-P from acidic soils whereas, less Ca-P from calcareous soil and failed to absorb Fe-P from any soil types. Inoculation with either of the two mycorrhizal fungi improved the uptake of Al-P by soybean in acidic soils, and also increased the uptake of Ca-P in calcareous soils and significant amount of Fe-P uptake was evidenced. These results suggested that AM can enhance uptake of fixed soil P. The efficiency rate of utilizing various forms of mineral P by mycorrhizal plants depends on the species of mycorrhizal fungi inoculated and soil types. Further, Chang and Young (1992) showed that inoculation of tea cuttings (cv. TTES No. 12) inoculated with AM fungi or PSBs significantly enhanced the growth of tea seedlings.

River sand mixed with chicken compost at a ratio of 4:1 (v:v) filled in Jiffypot and added *Glomus clarum* inoculant to have 50 AM fungus spores and then planted with melon. The result showed the flowering time could be 7 days shorter than that of treatment without adding the inoculant. In green house experiment, it showed the quality of melon could be increased. The percentages of first class and second class melons were increased by 22% and 52%, respectively. After harvesting its fruit, the melon's stolon stem was removed and the new bud was successfully developed to have 70% survival percentage. Within 35 days, melon fruit attained its maturity to have another harvest (Cheng and Chung 2001). The research result showed that such process could be used to lessen the cost of buying melon seedling.

Cheng and Chung, 2004 and Chiu *et al.* 2004 conducted an ecpreiment in papaya. Papaya seedling in pot medium containing 150 spores of AM fungi (*Glomus aggregatum*, *Glomus etunicatum*, *Glomus clarum*, *Glomus mosseae*, *Acaulospora scrobiculata*) grew faster and wider root system than those of control treatment. The shoot fresh weight and height of orange tree inoculated with AM fungus spore were 30.2-67.4% heavier and 17.6-39.4% higher than those of orange tree without inoculation after the experiment was carried out four months. Pot media planted with different flower crops added with AM inoculants have more than 100 spores has earlier flowering time, more numbers of flower, larger flower diameter, heavier fresh and dry flower weights than those of pot media without adding AM inoculants (Zhang 1990; Zhang, 1993; Zhang 1995; Wang 1997; Wang *et al.* 2004).

5. Using organic material degrading microbes to increase the available nutrient contents of organic fertilizer

Fresh weight of cabbage grown in pot medium made from pig compost was added to the protease bacterium suspensions (10^8 cfu.ml^{-1}) at a ratio of 1:100 (w:v) and mixed with vermiculite at a volume ratio of 1:9 (v:v) were 33-51% heavier than that of control treatment (Chien 2005).

Tsai (2005) developed an efficient way to process organic residues, rice straw, rice hull and sawdust, with *Trichoderma* sp. and *Bacillus* sp. to create special composts. The composts could have positive effect on the soil fertility and promote the growths of calla lily (*Zantedeschia* Hybrids) and rose (*Rosa* Hybrids Hort.).

Several protease bacteria were isolated from decaying organic debris, composts and soil. The suspensions of the bacteria inoculated at a rate of 1:100 to aqueous solutions containing 10% soybean or meat-bone powder for carrying out liquid fermentation. The results showed the available nitrogen concentrations of soybean powder and meat-bone powder aqueous solutions inoculated with protease isolate suspensions were several fold than those of non-inoculated treatments (Chien 2006).

FARMERS' ACCEPTANCE AND UTILIZATION OF BIOFERTILIZERS

In order to promote sustainable agriculture, both central and local government agencies in Taiwan are supporting extensive application of bio-fertilizers. Major programs for the application of bio-fertilizer include production of rhizobial, P-solubilizing microbial inoculants for soybeans that can be used as vegetables and for other crops, and production of AM-inoculants for melons and other horticultural crops. It also aims at improving biological nitrogen fixation in soybeans that are consumed as vegetables, peanuts, and in red bean. Similarly, emphasis in attaining higher yield and better quality horticultural crops were being given through three major programs- the production of inoculants, extension programs so that farmers can apply inoculants on to their farms and demonstration and awareness programs to show farmers the benefits of inoculated plots.

Soybeans for vegetable purpose are extensively produced in Taiwan and exported to Japan. Superior and constant maintenance of quality will be an important factor governing the export value of soybeans in the international market. But earlier, farmers were applying more chemical fertilizer than the recommended levels leading to inferior quality of beans. Since 1988, the Department of Soil and Environmental Sciences at National Chunghsing University in Taiwan actively started the production of efficient inoculants (liquid and solid bio-fertilizers) that can maintain yield and superior quality soybeans, which were exported and presently being consumed as vegetables in several countries. During last 20 years (from 1987 to 2006), enough inoculants were produced to inoculate approximately 65,091 ha of farmland. Over the years, farmer's economic gain also increased significantly (US$27 million) on using rhizobial inoculants. Moreover, a great deal of chemical fertilizer was saved and further groundwater pollution caused by N leaching was significantly reduced.

Healthy seedlings are one of the essential factors affecting the growth and yield of crops. Over the past decades, AM inoculants have been produced by the Agricultural Research Institute of COA, National Chunghsing University, National Pingtung University of Science and Technology in Taiwan, and the inoculants were distributed and technologically demonstrated to farmers by several Agricultural Experimental and Improvement District Stations for inoculating many crops, particularly horticultural and ornamental plants such as muskmelon, citrus, strawberry, lily, tomato, chrysanthemum, gerbera, tea, and fruit trees (Chang 1987 1993, 1994; Chang and Young 1992ab; Cheng and Chung 1991-2006; Chen and Hung 1994; Wang 2005-2007; Young 1990-2007).

FUTURE PERSPECTIVE OF BIO-FERTILIZERS IN TAIWAN

Excess nutrients are accumulated in soils, particularly P as a result of over application of chemical fertilizers by farmers during intensive agricultural practices. Hence, major research focus should be on the production of efficient and sustainable bio-fertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems. In view of overcoming this bottleneck, it will be necessary to undertake short-term, medium, and long-term research, in which soil microbiologists, agronomists, plant breeders, plant pathologists, and even nutritionists and economists must work together.

The most important and specific research needs should highlight on following points:

1) Selection of effective and competitive multi-functional bio-fertilizers for a variety of crops.
2) Quality control system for the production of inoculants and their application in the field, to ensure and explore the benefits of plant-microorganism symbiosis.
3) Study of microbial persistence of bio-fertilizers in soil environments under stressful conditions
4) Agronomic, soil, and economic evaluation of bio-fertilizers for diverse agricultural production systems.
5) Transferring technological know-how on bio-fertilizer production to the industrial level and for optimum formulation.
6) Establishment of "Bio-fertilizer Act" and strict regulation for quality control in markets and application.

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