USE OF BIOFERTILIZERS AND BIOPESTICIDES TO PROMOTE PLANT GROWTH AND NUTRIENT UPTAKE

Cheng-Hua Huang
Department of Soil and Environmental Sciences, National Chung Hsing University,
South Dist., Taichung City, Taiwan
E-mail: chhuang@nchu.edu.tw

ABSTRACT

Biofertilizers and biopesticides can be used to promote crop growth and health, which may partly replace the use of chemical fertilizers and pesticides. Of these biofertilizers and biopesticides, plant growth-promoting rhizobacteria (PGPR) play an important role in improving plant growth. Since microorganisms are considered as an important tool in overcoming problems related to the excessive use of chemical fertilizers and pesticides, microbial products have been extensively studied and will be widely used in the future. In this review, some examples of biofertilizers and biopesticides will be discussed based on our research conducted in Taiwan, including Azotobacter, Bacillus, bacterial endophytes, and Trichoderma. Appropriate use of biofertilizers and biopesticides is vital for developing sustainable agriculture.

Keywords: biofertilizer, biopesticide, mineral nutrition, plant growth-promoting rhizobacteria, disease control

INTRODUCTION

Chemical fertilizers have been extensively using to obtain high yields, but their intensive application has raised concern about groundwater pollution and greenhouse gas emission. It is necessary to develop ecofriendly agricultural practices for sustainable agriculture. Biofertilizers can enhance the nutrient status of their host plants via biological N₂ fixation, making nutrients more available, or increasing plant access to nutrients. Therefore, biofertilizers have become of paramount importance in food safety and sustainable crop production (Bhardwaj et al., 2014). Although a very wide open interpretation of the term biofertilizer, it is a contraction of biological fertilizer and should contain living organisms (Vessey, 2003). In addition, some biofertilizers have antagonist ability against plant pathogens and pests, which may also be used as biopesticides to improve plant health. Of these biofertilizers and biopesticides, plant growth-promoting rhizobacteria (PGPR) play an important role in improving plant growth by exploring multiple mechanisms such as increasing nutrient availability and uptake to the plant, facilitating plant protection, and enhancing stress tolerance (Tabassum et al., 2017). Many PGPR have been effectively evaluated for their performance in biological control and plant growth promotion such as Bacillus, Pseudomonas, and Burkholderia. Since microorganisms are considered as an important tool in overcoming problems related to the excessive use of chemical fertilizers and pesticides, microbial products have been extensively studied and will be widely used in the future (Vassilev et al., 2015). In this review, some examples of biofertilizers and biopesticides will be discussed based on our research conducted in Taiwan.

AZOTOBACER

Biological nitrogen fixation (BNF) can substitute some chemical nitrogen fertilizer use in rice cultivation because nitrogen-fixing bacteria can transform atmospheric nitrogen into fixed nitrogen used by plants (Choudhury and Kennedy, 2004). Rice is a monocot, non-leguminous crop, suggesting that associative N₂-fixing microbes may play a key role for in situ nitrogen fortification (Zaki et al., 2009; Sahoo et al., 2014). Of non-symbiotic free-living nitrogen-fixing bacteria, Azotobacter has been proved in fixing nitrogen for rice plants and promoting rice growth through releasing some beneficial compounds (Piao et al., 2005; Das and Saha, 2007; Sahoo et al., 2014). The genus Azotobacter belongs to the γ-subclass of the Proteobacteria and includes A. armeniacus, A. beijerinckii, A.
chroococcum, A. nigricans, A. pspali, A. salinestri, A. tropicalis, and A. vinelandii (Jiménez et al., 2011; Özen and Ussery, 2012). Azotobacter can fix at least 10 mg N/g carbohydrate and provide 19-47% of total nitrogen requirement in rice (Choudhury and Kennedy, 2004; Tejera et al., 2005), reducing the use of chemical nitrogen fertilizers. In addition to nitrogen fixation, Azotobacter can improve plant growth via increasing nutrient availability such as phosphorus and produce growth hormones such as IAA, gibberlins, and cytokinins, siderophores and antifungal compounds (Sahoo et al., 2014). However, environmental conditions influence the performance of BNF, suggesting that indigenous nitrogen-fixing bacteria may better adapt local niches than inoculant microorganisms and thus native strains would be more appropriate for use as biofertilizers for regional crops (Martinez-Toledo et al., 1985; Kannan and Ponmurugan, 2010).

Four species of Azotobacter including A. beijerinckii, A. chroococcum, A. tropicalis, and A. vinelandii were identified form rice rhizosphere soils in Taiwan (Chen et al., 2018). Of these four species, A. chroococcum was predominant (51.0%) but A. beijerinckii had the highest level of nucleotide diversity. Strains within individual Azotobacter species showed diverse profiles in carbon source utilization. In addition, a significant relationship was detected between the species diversity of Azotobacter and soil pH, Mn, and Zn. Members of the same Azotobacter species showed varying plant growth promoting traits, suggesting that these strains may not be equally effective to promote rice growth. Of 12 strains evaluated, A. beijerinckii CHB 461, A. chroococcum CHB 846, and A. chroococcum CHB 869 may be used to develop biofertilizers for rice cultivation because they significantly promoted rice growth. In addition to using indigenous Azotobacter strains, it is necessary to further determine the effects of combination of different Azotobacter strains with various plant growth-promoting traits on rice growth.

Azotobacter may also be used to promote amaranth growth, but it is better to apply Azotobacter with composts (Table 1). Although amaranth supplied with 20% chemical fertilizer reduction and inoculated with Azotobacter sp. R31 shows a comparable yield with the chemical fertilizer treatment, application of chicken manure compost and Azotobacter sp. R31 significantly increases dry weight of amaranth plants by 86.1% as compared to the chemical fertilizer treatment. These results suggest that combination of composts and Azotobacter can replace chemical fertilizers in amaranth cultivation.

Table 1. Effect of Azotobacter inoculation on dry weight of amaranth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizer</td>
<td>0.74c</td>
</tr>
<tr>
<td>Chemical fertilizer²</td>
<td>1.80b</td>
</tr>
<tr>
<td>Azotobacter sp. R31 alone</td>
<td>0.58c</td>
</tr>
<tr>
<td>20% Chemical fertilizer reduction + Azotobacter sp. R31</td>
<td>1.77b</td>
</tr>
<tr>
<td>Chicken manure compost + Azotobacter sp. R31</td>
<td>3.35a</td>
</tr>
</tbody>
</table>

*Values with different letters in the same column are significantly different as denoted by the LSMEANS statement of the GLIMMIX procedure in SAS v9.4 at the 5% level of significance according to Fisher’s least significant difference (Fisher’s LSD).

² The N fertilizer was provided as urea at 150 kg N ha⁻¹, P as single superphosphate [Ca(H₂PO₄)₂] at 105 kg P₂O₅ ha⁻¹, and K as potassium chloride (KCl) at 135 kg K₂O ha⁻¹.

³ Chicken manure compost was applied at a rate of 150 kg N ha⁻¹.

**BACILLUS**

The genus Bacillus represents a very large, diverse set of bacteria with the ability to form dormant endospores aerobically under unfavorable growth conditions (Fritzte, 2004). Several Bacillus spp. have been registered as biofertilizers and biopesticides, and Bacillus is the most common genus registered as phosphate solubilizing biofertilizers in Taiwan. Although tricalcium phosphate (TCP) has been used to isolate phosphate solubilizing bacteria (PSB) for biofertilizer registration, it may not be a sole selector because some PSB selected using TCP as an initial selection factor fail in promoting P uptake by plant roots (Bashan et al., 2013). We evaluated two commercial phosphate solubilizing biofertilizers made in Taiwan for their ability to promote plant growth and P uptake. Although commercial inoculant A (B. safensis) did not significantly increase dry weight of amaranth plants compared with the chemical fertilizer treatment, commercial inoculant S (B. subtilis) significantly increased dry
weight of these plants (Fig. 1). However, sterilized commercial inoculant S could still significantly increase dry weight of the amaranth plants, suggesting that nutrients rather than the bacterium in the inoculant might provide for plant growth. In contrast, sterilized commercial inoculant A significantly reduced amaranth growth, suggesting that B. safensis in the biofertilizer could promote dry weight of amaranth plants. Interestingly, sterilized commercial inoculant S significantly reduced P uptake in comparison with the unsterilized one, suggesting that B. subtilis in the biofertilizer had the ability to promote P uptake (Fig. 2). In contrast, sterilized commercial inoculant S did not significantly reduce P uptake in comparison with the unsterilized one. Therefore, B. safensis in the biofertilizer might not be a phosphate-solubilizing bacterium. Based on these findings, a thoroughly evaluation of both in vitro P-solubilizing ability and P uptake promotion on a model plant may be necessary to obtain potential PSB.

Apart from being used as biofertilizers, some Bacillus species have been registered as biopesticides in Taiwan such as B. amyloliquefaciens and B. subtilis. We evaluated the biocontrol efficacy of B. amyloliquefaciens CHB 310 against bacterial spot on tomato caused by Xanthomonas perforans. In comparison with the untreated control and the copper standard (cupric hydroxide + Mancozeb), foliar sprays of CHB 310 significantly reduced disease progress and the final disease severity of bacterial spot (data not shown). Although foliar applications of potassium silicate significantly reduced bacterial spot, foliar sprays of CHB 310 in combination with soil applications of potassium silicate also significantly decreased disease progress and the final disease severity of bacterial spot. Therefore, silicon fertilization may be used with B. amyloliquefaciens CHB 310 due to their synergistic effect in control of bacterial spot on tomato.

Fig. 1. Effect of phosphate solubilizing biofertilizers (PSB) on dry weight of amaranth plants.
A = commercial inoculant A (Bacillus safensis); B = commercial inoculant S (B. subtilis);
C = sterilized commercial inoculant A; D = sterilized commercial inoculant S;
E = NPK fertilization; F = NP fertilization.
Fig. 2. Effect of phosphate solubilizing biofertilizers (PSB) on P uptake by amaranth plants.
A = commercial inoculant A (*Bacillus* safensis); B = commercial inoculant S (*B. subtilis*);
C = sterilized commercial inoculant A; D = sterilized commercial inoculant S;
E = NPK fertilization; F = NP fertilization.

**BACTERIAL ENDOPHYTES**

Bacterial endophytes have been isolated from many plants studied, which can colonize the internal tissues of their host plant showing no external infection or negative impact (Ryan et al., 2008). Most endophytes originate from the rhizosphere or phyllosphere, while some may be transmitted through seed. Bacterial endophytes have been used to promote plant growth, control plant diseases, and remove soil contaminants. We evaluated three endophytes, *Pseudomonas* sp. CHB 1107, *Microbacterium* sp. CHB 1264, and *Variovorax* sp. CHB 1270, isolated from vegetables grown in Taiwan for their ability to alleviate salt stress in tomato plants. Seedlings of tomato plants were grown in a growing medium in which its electrical conductivity of a saturated paste extract (ECe) was adjusted to 1, 2, 4, 6, and 8 dS/m. In general, total dry weight of tomato plants was significantly reduced with increasing the ECe in the growing medium. Total dry weight was reduced by 31.5-40.0% as ECe was increased from 2 to 4 dS/m, independent of inoculation with or without the endophytes. Compared to the non-inoculated control, however, *Variovorax* sp. CHB 1270 significantly increased total dry weight of tomato plants grown in the ECe of 4 dS/m by 23.1% (Fig. 3). In addition, *Pseudomonas* sp. CHB 1107 significantly increased total dry weight of tomato plants grown in an ECe of 6 dS/m by 20.7% in comparison with the control. Although *Variovorax* sp. CHB 1270 did not increase total dry weight of tomato plants grown in an ECe of 8 dS/m, *Pseudomonas* sp. CHB 1107 and *Microbacterium* sp. CHB 1264 significantly increased total dry weight by 33.3% in comparison with the control.

Bacteria can secrete 1-aminocyclopropane-1-carboxylic acid deaminase (ACC-deaminase) to facilitate plant growth and development by reducing plant ethylene levels, especially following a variety of environmental stresses (Glick et al., 2007). Although *Pseudomonas* sp. CHB 1107 and *Variovorax* sp. CHB 1270 can secrete ACC deaminase, their ability to alleviate tomato plants under salt stress may vary probably due to their difference in plant growth-promoting traits and salt tolerance. *Microbacterium* sp. CHB 1264 cannot secrete ACC deaminase, but it can significantly increase total dry weight of tomato plants grown in an ECe of 8 dS/m, suggesting that the ability to secrete ACC deaminase is not an only selection factor for endophytes alleviating salt stress. Moreover, these endophytes alleviate salt stress depending on the application rate of nitrogen fertilizer (data not shown), suggesting that fertilization may influence the efficiency of these plant growth-promoting endophytes. Although soil management may affect the efficiency of endophytes, bacterial endophytes have the potential to develop sustainable systems of crop production.
Fig. 3. Effect of bacterial endophytes on total dry weight of tomato plants under salt stress. The electrical conductivity of a saturated paste extract (ECe) of a growing medium was adjusted to respective 1, 2, 4, 6, and 8 dS/m using NaCl.

TRICHOSTERMA

Isolates of several *Trichoderma* species have been reported to effectively reduce plant diseases (Srivastava et al., 2010; Marzano et al., 2013). The antagonistic interactions of *Trichoderma* spp. with different plant pathogens determine their biocontrol efficiency of the pathogens. Biocontrol mechanisms of *Trichoderma* species consist of antibiosis, mycoparasitism, competition for nutrients and potential infection courts, and induced systemic resistance in plants (Papavizas, 1985; Harman, 2006; Segarra et al., 2010; Druzhinina et al., 2011). However, the level of biocontrol of diseases caused by *F. oxysporum* using *Trichoderma* spp. may vary because a particular strain of *Trichoderma* expresses high levels of one or another antagonistic mechanism of action (Marzano et al., 2013). Therefore, it is necessary to select an effective strain and species of *Trichoderma* to control a given plant pathogen. Similarly, it is necessary to select appropriate strains of *Trichoderma* species for containerized-transplant production to obtain a superior biocontrol performance of Fusarium wilt of tomato since growing media have been used for planting tomato seeds before tomato seedlings can be transplanted into field soils (Jones et al., 1991). Once contaminated by pathogenic *Fusarium oxysporum*, the growing media can also be a inoculum source, and the pathogen may be further disseminated via wind, water, shoes, tools, and equipment (McGovern, 2015). Therefore, the growing media pre-inoculated with antagonistic *Trichoderma* species may enhance their suppressiveness to Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL).

We isolated *Trichoderma asperellum* CHF 78 from a commercial manure compost, and this strain also showed several plant growth-promoting traits including the ability to solubilize Ca$_2$(PO$_4$)$_2$, and to produce cellulases, chitinases, indole acetic acid (IAA), proteases, and siderophores (Li et al., 2018). In addition, CHF 78 significantly increased dry weight and plant height of tomato plants inoculated with or without FOL compared to those inoculated only with FOL. Inoculation of tomato plants with CHF 78 significantly reduced disease severity of Fusarium wilt, and pre-inoculation of tomato plants with CHF 78 followed by inoculation with FOL significantly promoted nutrient uptake of P, K, Mg and Zn as a result of reducing disease severity and these plant growth promoting traits. Interestingly, there was a significantly negative correlation between disease severity and nutrient uptake of all the elements analyzed. *Trichoderma asperellum* strain CHF 78 can potentially be used to reduce Fusarium wilt and...
promote plant growth and nutrient uptake under commercial tomato production.

Composts inoculated with beneficial microorganisms have been proved to increase crop production and reduce plant diseases (Ling et al., 2010; Liu et al., 2013; Zhang et al., 2013). Of these beneficial microorganisms, *Trichoderma*-inoculated composts have been used to effectively reduce disease severity of a number of plant diseases (Trillas et al., 2006; Blaya et al., 2013; Zhang et al., 2013). The higher degree of disease suppressiveness of the composts inoculated with *Trichoderma* may be due to not only its biocontrol ability, but also to its induced changes in both soil abiotic and biotic characteristics (Blaya et al., 2013). It has been suggested that inoculation of crops with *Trichoderma* species enhances nutrient uptake by plants (de Santiago et al., 2011). We investigated the possibility of using manure composts for organic amaranth cultivation under field conditions and determined the effects of the manure composts inoculated with (MC+CHF 78) or without *Trichoderma asperellum* CHF 78 (MC) on the yield and nutrient uptake of amaranth as well as soil properties. A field trial was carried out with four treatments including a control without fertilization, chemical fertilization (CF), MC, and MC+CHF 78 arranged in a randomized complete block design with six replications. MC and MC+CHF 78 treatments significantly increased the yield of amaranth by 96.2-102% in comparison with CF (Fig. 4). In addition, MC and MC+CHF 78 treatments significantly increased soil pH, organic matter, available P, exchangeable K, and microbial activity compared to the control and CF. Although MC and MC+CHF 78 treatments significantly enhanced N and K uptakes by amaranth plants, only MC+CHF 78 showed a significantly greater P uptake than that of the control (Fig. 5). The manure composts can be used as a complete substitute of inorganic fertilizers for organic amaranth cultivation under field conditions.

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 4. Effects of manure composts and inorganic fertilizers on the yield (fresh weight) (A) and dry weight (B) of amaranth. Control = no fertilization; CF = chemical fertilizer; MC = manure compost; MC+CHF 78 = manure compost inoculated with *Trichoderma asperellum* CHF 78.
Fig. 5. Effects of manure composts and inorganic fertilizers on uptake of N (A), P (B), and K (C) by amaranth. Control = no fertilization; CF = chemical fertilizer; MC = manure compost; MC+CHF 78 = manure compost inoculated with Trichoderma asperellum CHF 78.
CONCLUSION

Azotobacter may be used to promote crop growth, but it is better to apply Azotobacter with composts. Although Bacillus is the most common genus registered as phosphate solubilizing biofertilizers in Taiwan, some Bacillus biofertilizers may not have the ability to enhance P uptake by plants but to promote plant growth. It is necessary to evaluate in vitro P-solubilizing ability and P uptake promotion on a model plant to obtain potential PSB. In addition, Bacillus may be used as biopesticides for controlling plant diseases. Bacterial endophytes have the potential to develop sustainable systems of crop production because they can promote plant growth and health. Apart from controlling Fusarium wilt of tomato and enhancing nutrient uptake by tomato plants, Trichoderma asperellum may be used with composts to increase nutrient uptake and growth of amaranth plants. Appropriate use of biofertilizers and biopesticides plays a vital role in developing sustainable agriculture.

REFERENCES


