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Review

Biocontrol of *Fusarium* wilt of banana: Key influence factors and strategies

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Fusarium **wilt of banana, caused by** ***Fusarium oxyspoum*** **f. sp.** ***cubense*** **(Foc), is one of the most important and destructive diseases of banana, and is known to be a major biotic limiting factor for the development of the present banana industry. Biocontrol on the destructive disease, the use of antagonist as biocontrol agents (BCAs) against Foc, constitutes an effective option for the management of the disease. The effectiveness of biocontrol agents depends on a range of biological and physico-chemical factors, including the type and properties of the biocontrol agents, the obstacles to the initial colonization of antagonists, as well as the variation factors after initial colonization. Various strategies can be implemented to optimize the biocontrol efficacy, such as the use of endophytes from banana plants as BCAs (favorably** ***Bacillus*** **spp.), the development of water and nutrition retaining agent, the application of proper carrier for BCAs, the restoration of soil biodiversity, and combined management of nematodes disease and** ***Fusarium*** **wilt. In this review, elements affecting the biocontrol efficacy of Fusarium wilt are analyzed in detail, and strategies to promote the biocontrol effects are proposed. Besides, the concept of "post-indigenousness" and "post-indigenous microbes" were firstly suggested.**

Key words: *Fusarium* wilt of banana, biocontrol, *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder & Hansen, *Bacillus* sp., endophtic bacteria.

INTRODUCTION

Fusarium wilt of banana, also called Panama disease, is caused by *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder & Hansen, shortly as Foc (Stover, 1962). *Fusarium* wilt is known to be one of the most important and destructive diseases of banana, which is especially serious in the Central and South Americas, parts of Africa (Viljoen, 2002), Sri Lanka, Burma, Thailand, Indonesia and the Philippines, resulting in heavy enormous economic losses each year (Ploetz, 1994; Stover et al., 1987). In the recent ten years, banana plantation areas in China, influenced by Fusarium wilt, have decreased dramatically, especially in Guandong, Hainan, Fujian provinces.

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The fungus infects banana plants through the roots and invades the vascular tissue (xylem), causing external symptoms like gradual wilting, progressive yellowing of banana leaves (spreads from leaf margins, and from older leaves to younger leaves), eventual collapse at the petiole, and longitudinal splitting of the outer leaf sheaths in the pseudostem (Yin et al., 2011). The distinguishing internal symptoms of the disease is the typical discoloration of vascular tissues (in roots, corm, pseudostem, fruit stalk) varying from light yellow to dark brown, which appears first in the outer or oldest leaf sheath, then extends up to the pseudostem (Ploetz, 2006). Eventually, the disease leads to the death of banana plants.

The pathogenic isolates of Foc have been traditionally grouped into four physiological races based on pathogenicity to host cultivars under field condition (Fourie et al., 2009). *F.* oxysporum f. sp. cubense race 1 (Foc R1) attacks ‘Gros Michel’, ‘Lady Finger’ (AAB) and ‘Silk’ (AAB) varieties. Foc R2 infects cultivar ‘Bluggoe’ (ABB), while Foc R3 infects Heliconia spp. (a close relative of banana). And Foc R4 is able to attack cultivar Cavendish and all cultivars that are susceptible to Foc1 and Foc2 (Persley, 1987; Ploetz, 1990).

Banana production was once severely threatened by *Fusarium* wilt caused by Foc R1. The disease almost devastated the ‘Gros Michel’-based banana industry in the Central American and Caribbean during the mid-1900s (Ploetz, 1994). This adversity persisted until the introduction of Cavendish cultivars, which are unsusceptible to Foc R1 and Foc R2 in tropical banana-growing regions (Fernández-Falcón et al., 2003). However, the prosperity did not last long. The Cavendish cultivars had then been found highly susceptible to a new race of Foc, Foc R4 (Ploetz and Pegg, 2000). Economic losses worldwide are enormous. It brings to the spiteful reality that the banana industry is once again threatened by *Fusarium* wilt.

Fusarium wilt is destructive because it seriously hampers banana production and is difficult to manage. *F. oxysporum* are able to infect more than 100 plants, and are divided into more than 120 host-specific forms, known as formae speciales (Minerdi et al., 2008). *Foc*, generally considered to be one of the most destructive formae speciales of *F. oxysporum* (Ploetz, 1990), is soil-borne and has strong saprophytic ability. The pathogen can survive in the soil for several decades by producing spores (specifically, chlamydospores), which will re-infect the susceptible banana plants (Stover, 1962). This adds to the difficulty of disease management.

Till date, results from disease management studies have been disappointing. No effective and efficient control strategies available can satisfy the needs for the management of disease worldwide. Although, Huang et al. (2012) reported that Chinese leek-banana rotation is an efficient way for controlling banana *Fusarium* wilt, the method is not economical because Chinese leek is in low need in the market. Beside, crop rotation tends to have limited effectiveness once a disease outbreak occurs because of its soil-born nature and strong vitality (Fravel et al., 2003). Soil disinfection using chemicals or pesticides are not recommended due to environmental and human health concerns. And fumigated soils with methyl bromide can be re-infected in two or three years in fields with susceptible cultivars (Stover, 1958). Developing resistant banana cultivars may solve this problem but such efforts are progressing slowly (Stover and Buddenhagen, 1986; Hwang and Ko, 2004). Considering the urgency of the disease, a complementary approach for managing *Fusarium* wilt involves the biological control (or biocontrol), an important component of integrated disease management programs, specifically, in this case, is to use naturally occurring antagonists and active substances (viruses, bacteria, fungi, active substances of natural origin) as biocontrol agents (BCAs) for disease management.

When compared with other approaches, the use of BCAs is proved to be an ecologically safe strategy for disease management. However, the technology is still in its infancy. Enormous efforts should be still contributed to the development of effective and safe BCAs for comer-cial application. This review would focus on the elements affecting the biocontrol efficacy of *Fusarium* wilt, the points needed to be considered in the development of BCAs, and the corresponding strategies to improve the biocontrol efficacy, aiming to provide guidance for the biocontrol of *Fusarium* wilt and point the way forward for enhanced utilization of BCAs.

ELEMENTS AFFECTING THE BIOCONTROL OF FUSARIUM WILT AND CORRESPONDING STRATEGIES TO IMPROVE THE BIOCONTROL EFFICACY

It is a systematic work to use biological agents to control banana *Fusarium* wilt. In order to achieve ideal results, we should at least take into account the following elements: the type and properties of the biocontrol agents, the obstacles to the initial colonization of antagonists, as well as the variation factors after initial colonization.

The type and properties of the biocontrol agents

The first step to achieve effective biocontrol is to find the suitable potential biocontrol agents (BCAs). A primary consideration in the selection of antagonists as BCAs for field application is their type and properties, which directly or indirectly affect the biocontrol efficacy, the production process, the post-processing, as well as the storage and transportation. Production methods for biocontrol agents must be at low cost and yield viable, highly effective propagules of high concentrations. Also, these propagules, such as spores, must be amenable to long-term storage as dry preparations (Jackson, 1997).

Previous studies and experiences implied that *Bacillus* species serves as ideal candidates for viable BCAs (Yilmaz *et* al., 2005; Bertagnolli et al., 1996; Szczech and Shoda, 2004; Farhana et al., 2011; Govindasamy et al., 2011; Tan et al., 2013). *Bacillus* spp. strains are advantageous as BCAs because they are tolerant to adverse environmental stresses by producing endospores, such as heat and desiccation (Schallmey et al., 2004). At present, a variety of strains of *Bacillus* spp. have been extensively applied as BCAs against soil-borne plant diseases (Gurr et al., 2005), including Rhizoctonia (Yu et al., 2002) and *Fusarium* (Schisler er al., 2002; Sun et al., 2011), which have been proven to bring about high biocontrol efficacy. In addition, using *Bacillus* spp. strains as BCAs, the post-processing costs are easy to control, and the storage and transportation conditions needed could be easily fulfilled (Schallmey et al., 2004). On the other hand, the application of other microorganisms as BCAs, such as nonpathogenic *F. oxysporum* (Nel and Steinberg, 2006), *Trichoderma* spp. (Thangavelu et al., 2004), has been demonstrated to result in high cost of production, storage and transportation.

Obstacles to the initial colonization of antagonists

The second key point needed to be considered is the colonization of antagonistic microbes. The biocontrol efficacy of the BCAs largely depends on the ability of the antagonistic microbes to colonize the plant root and the rhizosphere, and to produce substances which inhibit pathogens. The colonization of antagonistic microbes is hampered by several natural barriers. Obstacles to the initial colonization refers to the first set of natural barriers encountered by the antagonistic microbes after the application of BCAs, including the predation and phagocytosis from soil protozoa (Ekelund et al., 2001; Ronn et al., 2002), inhibition from the exudates of indigenous microbes (Bolwerk et al., 2003) or plant roots (Chao et al., 1986), the competition with indigenous microbes for ecological sites, nutrients and energy.

Normally, influenced by these barriers, the population of most antagonistic microbes reduced drastically in the first 2 to 3 days after the application of the BCAs (Christoffersen et al., 1995). However, BCAs usually must maintain a certain population of the antagonistic microbes to obtain acceptable levels of disease suppression (Wang et al., 2011). Thus, in order to achieve satisfactory biocontrol efficacy, appropriate measures should be taken to help the antagonistic microbes get through this hard time. One of the alternatives is to increase the original population of the antagonistic microbes in the BCAs, ensuring that a considerable part of the population survive this adverse phase under the regulation of environmental factors. Another recommendation is to apply BCAs repeatedly to maintain certain levels of the antagonistic microbes. Repeated applications of BCAs can be achieved by distributing the BCAs through the irrigation water.

Predation and phagocytosis from soil protozoa

Predatory protozoa in the soil to some extent act as the regulator of the population of soil bacteria and fungi (Bird et al., 2003; Ingham et al., 1985). They prey selectively on certain bacteria and fungi, thus exert an effect on the population and diversity of their prey (Burke et al., 2003). The antagonistic microbes in the BCAs, of course, are affected inevitably by this regulation. One proposed solution to counteract this regulation is to inoculate the BCAs directly into the leaves or stems of banana plant through drip injection, but not to apply them into the soil. Specifically, the approach means to inject the BCAs intermittently into the plant vessel from up to down under the law of gravity, and let them spread into the cell eventually. However, one leading problem of this method is the increased costs. Advanced techniques are needed to be developed in order to reduce the costs to an acceptable level for large-scale field applications.

Besides, studies have shown that the spatial heterogeneity of soil may have something to do with the stability of the predator-prey relationship, since certain soil structures are beneficial for the survival of bacteria, for example, in the case that the spaces in the soil are suitable for bacteria to live in but are inaccessible to protozoa (Wardle, 2006). Soil aggregates are clumps of soil particles that are held together by moist clay, organic matter (like roots and fibrous roots), gums (from bacteria and fungi) and by fungal hyphae. Study revealed that well-aggregated soil is thought to be a very desirable habitat for bacteria (Wardle, 2006). Bacteria tend to accumulate inside soil aggregates because this environment provides them with micro-ecological sites that are not accessible to certain protozoa, that is to say, bacteria in this environment are less likely to be eaten by soil animals like protozoa and mites. Such knowledge gives us some clues on the application of BCAs- to make the carrier of BCAs into the structure of soil aggregates. This approach can to some extent protect the antagonistic microbes from the prey of indigenous predatory protozoa, thus conserving a substantial number of the microbes as a result. One recommendation currently, is to apply resin-coated controlled-release materials as the carrier of BCAs (Ko et al., 1996; Lunt, 1968).

Inhibition from the exudates of indigenous microbes or plant roots

A substantial amount of studies have reported that certain exudates of indigenous microbes and plant roots exert an inhibitory effect on heterologous microorganisms (Quintana et al., 2009; Bais et al., 2006; Hirsch et al., 2003). In addition, plants tend to develop an induced systemic resistance to heterologous microorganisms (Pieterse et al., 1998). These effects exert strong negative influences on the colonization of the heterologous antagonistic microbes in banana plants. To overcome these difficulties, a proposal is to screen active antagonistic microbes from the endophytic strains of healthy banana plants (Lian et al., 2008). The use of endophytes as antagonist is of great interest because of their ability to colonize plant tissue and produce antibiotics *in situ* (Sessitsch et al., 2004).

Of course, the endophytes isolated from a certain plant tend to be plant tissue and variety specific. That is to say, the endophytes from one cultivar might not be endophytic to another cultivar due to genetic diversity, which may stand in the way of the colonization process. In fact, the variety of banana cultivars in China is relatively uniform geographically, mostly Brazil and Williams, both belonging to *Musa* AAA group. Normally, it would not be difficult for the endophytes to colonize the varieties from the same group. Our recent studies have preliminarily confirmed the feasibility of endophytes (Wang et al., 2011).

When the BCAs have to be applied into soil, to overcome the inhibition from indigenous microbes and plant roots, or the “induced systemic resistance” mentioned above, a recommended option is to introduce a cover for the antagonistic microbes, which shelters the microbes from antimicrobial agents and environmental stress by acting as a physical barrier. For example, to entrap the biocontrol agents in capsule-like materials (Candela and Fouet, 2006) or coated and controlled-release materials mentioned above. The advantages of entrapped BCAs include: a controlled release of agent (controlled by the environment and the properties of the entrapment materials); easy handing and prolonged shelf life; protection against extreme environmental conditions.

The competition with indigenous microbes for ecological sites, nutrients and energy

The colonization of the antagonists is also impacted tremendously by indigenous microbes through competition for ecological sites, nutrients and energy (Hyakumachi, 2000). The competition, creating a nutrient-limiting environment, determines how different microbial populations coexist in the same ecosystem. Competition for essential resources is a factor that determines the survival of all organisms (Hyakumachi, 2000). The biocontrol fails if the antagonistic organisms are out-competed by other indigenous microbes in the soil for a limited essential resource. Thus, the antagonists must have the ability to use energy substrates and nutrients at low concentrations. Studies revealed that strains of *Pseudomonas fluorescens* and *Trichoderma harzianum* showed antagnostic effect on Foc. (Rujappan et al., 2002; Saravanan et al., 2003).

Obstacles to the long-term colonization of antagonists: The variation factors after initial colonization

The variation factors after initial colonization means the factors that affect the long-term colonization of antagonists. *Fusarium* wilt is a typical soil-borne disease. To achieve ideal biocontrol efficacy, the antagonists must colonize the rhizosphere soil and sustainedly occupy favorable ecological sites in the environment. Previous studies have illustrated that the long-term colonization of antagonists is correlated to soil moisture (or the water content of soil), temperature, soil aeration, pH, soil salinity, as well as the continuous supply of nutrients (Sessitsch et al., 2004; Bashan et al., 1991; Bevivino et al., 2005; Cavaglieri et al., 2005; de-Bashan and Bashan, 2008).

According to latest studies, rhizosphere bacteria exist not in the traditional recognized status (planktonic cells), but in biofilms (Webb et al., 2003). A biofilm is “a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface” (http://en.wikipedia.org, 20090205), formed by an aggregate of microorganisms in which cells adhere to each other and/or to a surface. In nature, biofilms constitute a protected growth modality allowing bacteria to survive in hostile environments (Rinaudi and Giordano, 2010). In this case, we believe biofilm is the best mode for antagonists to occupy heterogeneous habitats, acting as a physical barrier sheltering the antagonists from antimicrobial agents and environmental stress. Thus, favorable conditions should be provided to facilitate the formation of biofilm in practice. As indicated by Chang and Halverson (2003), the formation of rhizosphere bacterial biofilm is mainly affected by water moisture, nutritional status and environmental parameters.

Soil moisture

One of the most significant requirements for biofilm forma tion is sufficient moisture. In banana plantations, soil moisture affects the structure of soil aggregates, and is significantly related to soil aeration, temperature, entropy and pH. Banana plants need plenty of water and fertilizer to grow (Goenaga and Irizarry, 1998), due to their large stems and leaves, shallow roots, rapid growth and high yield. They do best in areas of high humidity (50% or more) and require regular applications of fertilizer. The growing periods and yield of banana plants depend largely on the rational management of fertilizer and water. In China, areas under banana cultivation are mostly dry slopes, platforms, plateaus, or plains in short of water. Irrigation in these areas is normally not convenient (Xia, 2011). The large-scale banana plantations are mostly watered through well irrigation, ditches-guided irrigation, showering irrigation via perforated pipe, or microjet irrigation (Chen, 2001). These irrigation means, although solve the water shortage problem to some extent, are far from enough to satisfy the sustained demand of banana plants for continuous water supply, especially in the vegetative stage, flower bud differentiation stage, flowering and fruit ripening stages. Besides, the irrigation means mentioned above are all based on high costs, which tend to benefit the large-scale plantations owners more and are relatively uneconomical to the smallholders.

Banana plant is also vulnerable to water logging, and the soil aeration condition directly determines the growth status of its root system (Goenaga and Irizarry, 1998). Waterlogging reduces yield and plant size, restricts root growth, causes shallow root systems, and stops the active uptake of nutrients (Aguilar et al., 2008). In a nutshell, banana plant is vulnerable to both blood (waterlogging) and drought. In this dilemma, most plantations have difficulty in maintaining a balanced water supply throughout the whole growing season, which inevitably results in the intermittent shortage of water. This problem will, undoubtedly, lead to changes in soil microbial communities, mostly, dramatic decrease in the population of soil microbes (which are irreversible for at least one growing season). This is the fundamental reason why most BCAs do not have a sustained effect in practice, according to our continued investigation in the recent 4 years (Guo et al., unpublished data).

Sustained water supply in rhizosphere soil leads to favorable rhizosphere environments for the biofilms formation of antagonists. Through biofilms, the population of antagonists in the rhizosphere can maintain a balance for quite a long time, which in turn prolongs the persistence of the colonization of the of antagonists. In conclusion, from our point of view, the population of antagonists is mostly dependent on the stability of sustained water supply in soil. In standard large-scale plantations, this demand can be satisfied through microjet irrigation and drip irrigation facilities. However, in small plantations, where it is not profitable and feasible to set up these facilities, a recommended option is to apply water retaining agents in rhizosphere soil.

There are two types of water retaining agents nowadays, one is chemical and the other is bioactive. Poly-γ-glutamic acid (Zeng et al., 2013), in short γ-PGA, act as a new kind of bioactive water retaining agent in agricultural and environmental applications. It is a biodegradable, water- soluble amino acid polymer with a molecular weight ranging from about 10,000 up to 2 millions, generated by the microbial fermentation in nature (Zeng et al., 2013). It is normally composed of about 5,000 glutamic acids molecules or mono units, with a free carboxyl group on the α-carbon atom of each repeating unit (Do et al., 2011).

Because of the repulsion of the negatively charged carboxyl, the chain space is extended to be particularly large. The interaction between molecules is strong even when γ-PGA is in low concentrations, which makes it an ideal material for super absorbent (Candela and Fouet, 2006). Studies have revealed that γ-PGA can effectively absorb soil water, and can reach a maximum water absorption coefficient of 1108.4 (Tsujimoto et al., 2010). Due to its nature of economical costs, biodegradability and water absorption, γ-PGA, as a water retaining agent, can be used to change soil aggregates structure, maintain soil moisture, and conserve fertilizer in soil. In our study, we have found several γ-PGA-producing strains that are antagonistic against Foc (Guo et al., unpublished data) under the application for a patent. Further studies are still under way to screen more efficient strains. Undoubtedly, the application of these bacteria as BCAs would bring more efficiency.

Nutritional status and environmental parameters

Nutritional status and several environmental parameters are also key factors that influence the formation of biofilm (Pan et al., 2010), which further affect the persistence of the colonization of the antagonists, including carbon source, amount of nitrate, phosphate, calcium and magnesium as well as the effects of osmolarity and pH (Rinaudi et al., 2006).

Availability of nutrition in the form of glucose results in increased biofilm formation of bacteria (Shera et al., 2006; Revdiwala et al., 2012). Increased levels of phosphate, Ca^{2+}, Mg^{2+} enhance biofilm formation, whereas osmotic agents, such as NaCl and sorbitol negatively affect biofilm formation through an osmotic effect as their concentrations increase (Rinaudi et al., 2006). Besides, temperatures and pH are also factors that affect biofilm formation (Pettit et al., 2010), and the effects differ from one bacterial species to another. Taken together, the nutritional and environmental requirements for biofilm formation appear to be rather species specific. Thus a specific antagonist should be tested for the optimal nutritional conditions for the biofilm formation, according to which various sugars, osmotic agents or salts should be supplemented to the medium for cultivation to provide feasible conditions for biofilm formation.

Currently, most banana plantations are in the condition of imbalanced soil nutrition (such as redundant nutrition in rhizosphere) and improved salinity (due to years of continuous cultivation and preference in employ of N, P fertilizer) (Zhong et al., 2011), which stand in the way of biofilm formation. Inadaptability to these conditions would definitely result in the rapid decline in the population of the antagonists, thus weakening the biocontrol efficacy.

The development of time release technology, also known as sustained-release (SR), provides an alternative for this problem. The BCAs are recommended to be introduced into a controlled-release system with sustained release of nutrition, for example, the water retaining agent or the slow-release fertilizers. In this system, the nutrients are provided slowly and steadily for an extended duration, which not only protect the antagonists and facilitate the biofilm formation, but also put banana plants in the way they prefer to be fed and helps them grow well. Considering the water shortage problem, it is desirable to develop a water and nutrition retaining agent to maintain both water and nutrition at the same time. In addition, as mentioned above, high salinity exerts negative influence on the formation of biofilms through an osmotic effect. A recommended approach is to use halo-tolerant microbes from mangrove and marine source as antagonists against Foc pathogens (Xu and Dai, 2007).

OTHER FACTORS THAT HELP IMPROVE THE BIOCONTROL EFFICACY AGAINST *FUSARIUM* WILT OF BANANA

Pay attention to the evolutionary and geographic diversity of Foc

By comparing DNA sequences of nuclear and mitochondrial genes, O'Donnell et al. (1998) concluded that *Fusarium* wilt of banana is caused by pathogenic strains with independent evolutionary origins, and the Foc races isolated are geographically distinct. A recent field experiment conducted by us supports this conclusion as well, which found that a biocontrol agent proved to have excellent effect in a field in Ledong, Hainan (having a control effect of 99%) exhibited poor performance in the field just 17 km around (having a control effect of 23%). Preliminary analysis revealed that the colony morphology and virulence of Foc R4 in the two fields showed significant difference (Mo et al., 2013). Therefore, the evolutional and geographic diversity of the pathogen should be taken into consideration in the screening of antagonists against Foc. In the first screening round, it is recommended to use pathogens from various evolutionary origins and geographic areas as targets, in order to improve the biocontrol efficacy.

Take into account the integrated management of banana nematodes

Banana nematodes constitute another major threat to banana production all over the world, which cause yield losses of up to 30 to 60% in many countries (Roderick et al., 2012). The fundamental reason that leads to nematode disease and *Fusarium* wilt is basically in agreement with previous studies (Zhong et al., 2011; Palomares-Rius et al., 2011), both due to the loss of biodiversity in soil. Banana plants that are infected with nematodes tend to have enhanced susceptibility to *Fusarium* wilt, even in those lines showing *Fusarium* tolerance or resistance (Ammar, 2007). Thus, the two diseases could be controlled jointly, and combined management of the two diseases would save us lots of efforts and costs (Pararu et al., 2009). To improve biocontrol efficacy, BCAs against both disease are encouraged to be developed.

Consider addition of non-antagonistic microbes into the biocontrol agents

Microbes that do not have antagonistic effect to pathogens may act as potential biocontrol agents by occupying the ecological sites of the pathogen or competing with the pathogen for nutritious and infection sites (Nel et al., 2006). Therefore, it is recommended to add certain non-antagonistic microbes into the biocontrol agents. Non pathogenic *F. oxysporum* strains can be developed as biocontrol agents. They are able to compete for nutrients in the soil, which affect the rate of chlamydospore germination of the pathogen (Kidane and Laing, 2010). They can also compete with the pathogens for infection sites on the root, and can trigger plant defense reactions, inducing systemic resistance. To give an example, Fo47, a non-pathogenic *F. oxysporum* strains, have been successfully applied as biocontrol agent in the field. Studies revealed that the general pattern of colonization in soil was similar for the pathogenic strain and Fo47. However, Fo47 grew faster than the pathogenic strain and, as a consequence, colonized the rhizosphere earlier (Michielse and Rep, 2009).

Considering using secondary metabolites for biocontrol agents

Some antagonistic microbes produce antagonistic substances only under the nutrient-poor conditions (Opelt and Berg, 2004). Based on our experience, this kind of microorganisms cannot act as viable biocontrol agents directly (Mo et al., 2013). A better choice is to take advantage of its secondary metabolites. If they are used as potential viable biocontrol agents, relatively poor culture conditions should be applied in the dual-culture assay during the second screening round, for example, cultured in the water agar. Only the microbes that exhibit antagonistic characteristics in the water agar should be chosen for the third screening round.

Avoid using actinomycetes as viable biocontrol agents

Actinomycetes is not recommended to be applied as viable biocontrol agents for managing *Fusarium* wilt of banana in Chinese banana plantations, because the favorable conditions needed for actinomycetes to survive is not desirable for the growth of banana roots (Jayasinghe and Parkinson, 2008). However, it is feasible to use the secondary metabolites of Actinomycetes as biocontrol agents.

Introducing limitation factors as many as possible in the screening of antagonists

In the second round of screening for potential BCAs, it is recommended to introduce limitation factors as many as possible, such as extreme pH and temperature. The antagonistic stains that are tolerant to a wide range of environmental conditions have greater market potential as biocontrol agents against Foc. In other words, the advantageous and competitive microbes have greater chance to colonize heterogeneous habitats and become “indigenous inhabitants” in this environment. The process for the microbes to become “indigenous inhabitants” in heterogeneous habitats is defined by us as “post-indigenousness”, and these microbes are designated as “post-indigenous microbes".

As we all know, the most typical feature of strains belonging to *Bacillus* family is that they can form spores to get through adverse environmental conditions (Vos et al., 2009), which enable them to survive in a board range of environmental conditions. The utility of strains of *Bacillus sp*. as biocontrol agents cannot only obtain sustained colonization in banana plants, but also facilitate the post-processing, storage and transportation. Therefore, antagonistic strains from *Bacillus* spp. are a recommended choice as biocontrol agents in field application.

Restoring the soil biodiversity in banana plantations to control *Fusarium* wilt indirectly

The most serious problem for banana plantations with years of continuous cultivation is the loss of soil bio diversity (Zhong et al., 2011). Especially, the loss of keystone species in the microbial communities would severely destroy the ecological balance of the environ ment (Walker, 1992), which offers favorable conditions for the accumulation of Foc pathogens. The toxins secreted by these pathogens first attack the roots of banana plants, and then spread to stem and leaves, eventually resulting in outbreak of *Fusarium* wilt (Kuo and Scheffer, 1964). The growth of banana plants is closely related to the biodiversity in the rhizosphere. Under constant supply of normal nutrition, the roots of the banana plants are well developed. The exudates from the root hairs or root tip promote the growth of rhizosphere organisms (Bais et al., 2006), which in turn enhance the biodiversity of soil.

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