การควบคุมโรคพืชโดยการใช้จุลินทรีย์ปฏิปักษ์

Plant Disease Control Using Antagonistic Microorganisms

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บทคัดย่อ

การควบคุมโรคพืชโดยการใช้จุลินทรีย์ปฏิปักษ์เป็นวิธีการหนึ่งในการควบคุมทางชีววิธีที่ประสบความสำเร็จในการนำไปใช้ โดยกลไกการทำงานของจุลินทรีย์ในการควบคุมโรคพืช ได้แก่ การเป็นปรสิต การผลิตสารปฏิชีวนะ การแก่งแย่งพื้นที่ การแก่งแย่งอาหาร การชักนำให้พืชสร้างความต้านทาน และการส่งเสริมการเจริญเติบโตของพืช นอกจากนี้ในบทความยังได้กล่าวถึงการประยุกต์ใช้จุลินทรีย์ปฏิปักษ์ รวมถึงข้อดีและข้อจำกัดในการใช้จุลินทรีย์ปฏิปักษ์ในการควบคุมโรคพืชด้วย

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Abstract

Plant disease control using antagonistic microorganisms is one biological control that has successfully been used. The mechanisms of these microorganisms in eliminating plant diseases include parasitism, antibiosis, competition for space and food, induced systemic resistance and induced growth response. Moreover, the applications, advantages and disadvantages of these antagonistic microorganisms will be reviewed.

Keywords: biological control, antagonistic microorganisms, plant pathogens

Introduction

Crop losses are caused by several kinds of problems including diseases, insects, and weeds. Plant diseases are one of the important causal agents. According to Agrios[1] and Cook[2], the effective control methods are using chemical pesticides and cultural practices. However, the increasing use of pesticides in crop production has been a point of public concern because they harmfully affect the environment and non-target organisms[1,2]. Moreover, some plant pathogens develop in such a way so as to resist the available pesticides and some pesticide companies hesitate to create and test new pesticides because of the difficulties of the registration process and the limited budget[2]. Therefore, the plant diseases control using non-chemical pesticides such as biological control is a very important and interesting topic to discuss.

Biological control is a possible alternative strategy to manage plant diseases. It uses one organism known as antagonistic microorganisms to inhibit growth and infect another organism or plant pathogen. This control is an environmentally friendly method[2,3]. Some plant pathogens that have successfully been controlled by these microorganisms are *Erysiphe* sp., the causal agent of powdery mildew disease; *Botrytis* sp., the causal agent of grey mold disease; and *Rhizoctonia* sp., the causal agent of damping off disease[1,2,4].

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Although, there are many researchers who study the antagonistic microorganisms and employ them in plant disease management, a clear understanding of these microorganisms for further development and effective control is required. The aim of this review is to first; describe the cause of plant disease and their significance; second, describe the definition of the antagonistic microorganisms and their mechanisms in plant disease control, Third; describe the research and application of the antagonistic microorganisms and forth; discuss the advantages and disadvantages of the antagonistic microorganisms.

1. Cause of Plant Disease

Any cause capable of harmfully affecting healthy plants may be considered as a plant disease. The main agents engaged in plant disease are illustrated in Figure 1. The cause of diseased crops may be one or more causes. A practical difference can be depicted between biotic and abiotic agents of disease. A lot of biotic agents, with the microbial pathogens, the parasitic plants and a few of the animal pests, are infectious. By reason of their ability to grow, reproduce and spread, these agents increase or spread from one host plant to others. In specific appropriate conditions, they may expand rapidly over broad regions[5].

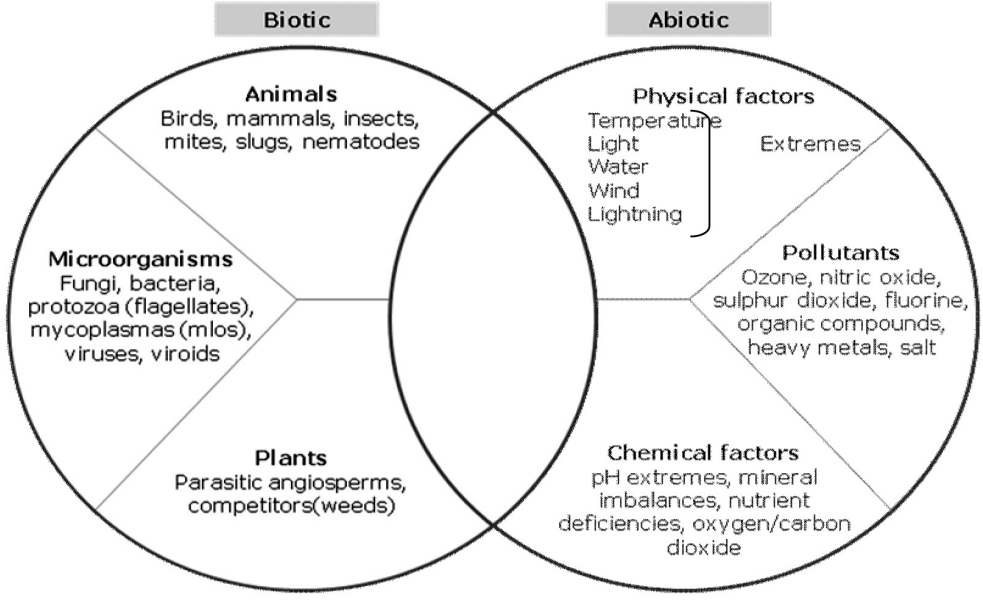

Figure 1. Agents responsible for plant diseases, disorders and damage.

Source: Lucas[5]

The causes of plant disease are very similar to the causes of human and animal disease. They consist of pathogenic microorganisms, for instance viruses, bacteria, fungi, protozoa and nematodes and inappropriate environmental circumstances, for example lack or excess of nutrients, moisture and light and the occurrence of toxic substances in the environment. Plants also become sick during competition with weeds and they are frequently destroyed by insects. However, the damage caused by insects, humans or animals is not considered to be a plant disease[1].

2. Significance of Plant Disease

In crop production, one of the causes affecting crop yield and quality is disease. Farmers and people who work in agriculture pay attention only to those modifies in crop performance that have an effect on cash earnings per production area. The ideal condition, in which plant pathogens are prevented, prohibited or eliminated, is an imaginary rather than a workable purpose. Therefore, the selection of crop variety is mostly derived from expected productivity rather than resistance or tolerance to disease. As a result, the

impact of a disease will rely on a significant limitation on the market cost of the product. Using chemicals or pesticides to control diseases are only necessary when the expected effect on crop or quality will outweigh the cost of the amount though agrochemical companies as well as affect decision-taking by advertising sales of their products. As the promoting force persuades a grower to use chemicals, the method currently accepted is more carefully considerated than in the past, and pesticide use is inhibited in circumstances where environmental side-effects might occur[5].

3. Disease Control

Over the last few decades, plant pests control has relied progressively on the widespread application of pesticides. Plant diseases management regularly requires the use of toxic chemicals on crops, on their products and into soil where numerous plant pathogens reside and damage the plant roots. Several chemical pesticides have been proved that they are toxic to non-target organisms including beneficial microorganisms, animals and humans. It is hard to assess the short- and long-term effect of environmental contamination from the result of our efforts to control plant disease on being health and welfare. A large amount of current research in plant disease control aspires to discover additional environmentally friendly methods for plant disease management. The majority of the methods consist of conventional plant breeding and genetic engineering of disease-resistant crops, the use of cultural practices to suppress plant disease and the application of antagonistic microorganisms to inhibit plant pathogens[1].

With regard to the challenges to crop production, the greatest ones are the decrease in the food loss resulting from plant diseases, the simultaneous increase in food quality and the improvement in the protection of the environment. Although, the global population is progressively increasing, many natural resources are gradually decreasing[1]. Therefore, it is essential to establish successful and safe control of plant disease in order to provide high drop yields and quality.

4. Definition of Antagonistic Microorganism

Biological control is becoming more prominent and is gaining popularity because of the concern for the effect of pesticides on non-target organisms and the pesticides contamination of the environment. The terms of 'biological control' or 'biocontrol' in plant pathology refer to the use of antagonistic microorganisms to inhibit diseases. Biocontrol needs the introduction and establishment of this microorganism in the plant pathogens community on or near the plant surface[2,3,6].

An antagonist has been broadly defined as an opponent or an adversary. In terms of biology, it refers to an associate of an interaction that interferes with another individual, ranging from interference among molecules to that among higher plants. In the case of plant disease control, antagonists are biological agents called antagonistic microorganisms with the ability to interfere in the life development of plant pathogens[2].

5. Mechanism of Disease Control

The mechanisms of antagonistic microbial biocontrol of plant pathogens include many modes. The most effective ones are antibiosis, competition, parasitism, induced systemic resistance, hypovirulence and induced growth response. These mechanisms are described below

5.1 Antibiosis

As a term in biology, antibiosis is an association between two or more microorganisms that is harmful to at least one of them. The success of a target microorganism's deterioration is that microorganisms named antagonistic microorganisms produce metabolic compounds with antibiotic activity which can directly inhibit, poison or kill target microorganisms. This association can be observed as a zone of inhibition appearing between two microorganisms while growing together on agar plates. There is abundant evidence and reports showing that some antibiotics produced by microorganisms are mainly successful against plant pathogens[6,7,8]. In addition, Whipps[9] reports that bacteria produce antifungal compounds including ammonia, HCN, Oligomycin A, Oomycin A, pyrrolnitrin (Pln), phenazine-1-carboxylic acid (PCA) and others. Several studies have revealed that some of them have

a broad-spectrum activity. For instance, pyrrolnitrin produced by bacteria *Pseudomonas* and *Burkholderis* species has presented activity against a variety of plant pathogenic fungi such as Basidiomycetes, Deuteromycetes and Ascomycetes[10]

5.2 Competition

As a biological term, competition is an interaction between organisms, in which the ability of one is decreased with the occurrence of another. The shortage of at least one resource such as food, water or space can cause competitive interaction between antagonistic microorganisms and plant pathogens (Begon et *al*., 1996). In soil, the nutrients and rhizosphere are regularly not adequate for all microorganisms. They have to successfully compete for their available food and territory. Consequently, they successfully colonize the phytosphere and the rhizosphere. In addition, antagonistic microorganisms are able to competently consume essential resources and they can efficiently fight with the plant pathogen. Many plant pathogens need such nutrients to germinate, then penetrate and infect the plant host tissue successfully. It is widely believed that competition for limited nutritional factors may be the occasion of the biocontrol of plant pathogens[3,6,12]. Additionally, it is also considered that such interaction is more significant for soil borne pathogens including *Fusarium* and *Pythium* species[12].

It has been revealed that non-pathogenic microorganisms usually protect the plant by rapid colonization. Hence, the limited available substrates or nutrients are not accessible for plant pathogens to grow up. For instance, fungi *Pythium ultimum*, the causal agent of damping off disease, is suppressed by bacteria *Enterobacter cloacae*[6,13,14].

5.3 Parasitism

Parasitism is an interaction in which one organism, the parasite, gains the resources of another organism or the host. The host is damaged by the interaction while the parasite benefits. As a term of biology, hyperparasitism, direct parasitism and interfungus parasitism are applied in indication of the occurrence of one parasitizing another. Hyperparasitisim comprises various interactions as well as slight and main morphological disorders, the overgrowth of hyphae of one fungus by another, penetration and direct parasitism by the formation of haustoria, and the lysis of one hyphae by another[15].

For example, *Trichoderma harzianum*, a parasitic fungus, has been revealed to penetrate the resting spores and hyphae of the arbuscular mycorrhizal species, *Glomus mossease*, resulting in dissolution of the hyphal and spore cytoplasm. Moreover, metabolites produced by this fungus may also play a part in the interaction of mycoparasitism because they change in spore protoplasm and cell wall integrity which is a phenomenon appearing prior to the penetration of the antagonists in plant pathogens. Consequently, it causes the death of spore or hyphae of *G. mossease*[16].

5.4 Induced systemic resistance

In terms of inducing plant disease resistance, the resistance mechanism of plant is consisted a short-term and long-term response. In a short-term response, a host plant is responded by pathogens which are in adjacent cells of the infection site and induce cell apoptosis to restrict a colonization of the pathogens[17]. The term of “Long-term resistance”, “induced systemic resistance (ISR)” or “systemic acquired resistance (SAR)”[18] has been used to describe the phenomenon of induced resistance after Chester[19] proposed the term “acquired physiological immunity”. In particular, the long-term response is focused as describe below.

Induced systemic responses (ISR) are broad-spectrum resistance to plant pathogens[20]. Mechanism of ISR is induced by prior treatment with either a biological or chemical agent which causes a damage of host plant tissue and an induced resistance signal respectively. The signal from the infection sites is translocated through the phloem in order to change or induce systemic immunity within the plant with the aim of defending the pathogen invasion[21]. The signaling molecules have been reported in the last few years, which including methyl salicylate, jasmonic acid, undefined glycerolipid derived factor, and a peptides that is involved in cell-to-cell basal defense signaling[20, 21].

For example, *Trichoderma harzianum* was used as biotic plant inducers for anthracnose pathogen resistant

in chilli. This biotic agent, 10^8 spore/ml of *T. harzianum*, showed 24.2 and 57.4% reduction of the infection percentage on seedling and mature stages, respectively[22]. In addition, lipopolysaccharides, siderophores and salicylic acid are produced from plant growth promoting *Pseudomonas* spp. which has successfully been used as biotic inducer for plant protection. The plant growth promoting *Pseudomonas* spp. induces cell wall structural modifications and biochemical/physiological changes that involved to plant defense mechanisms[23].

5.5 Hypovirulence

As a biological term, hypovirulence is a pathogenic strain reduced on virulence level because it has infected with an infectious agent or hyperparasite. Hypovirulence as a natural incident that limited the spread of chestnut blight disease was first reported in Italy[24]. This limiting is caused by the effect of viruses in the species *Cryphonectria hypovirus1* (family *Hypoviridae*) on the fungal, *Cryphonectria parasitica*. Moreover, the hypovirulence was found that it is a fungal characteristic, maternal inherited, and transmissible by fungal anastomosis[25]. Therefore, this hypovirulence was potentially used for biocontrol of chestnut blight[26].

5.6 Induced growth response

To enhance plant growth, one of the significant inducers is rhizosphere microorganism[27]. Some of rhizosphere microorganisms have an antagonistic property so they not only can be used as a plant growth promoting microorganism for increasing the plant growth but also can be used as a biological control agent. Such as *Saccharomyces unispora* and *Candida steatolytica*, they have an antagonistic characteristic to *Fusarium oxysporum* and they were able to increase kidney bean growth compared to infected and non-infected plants[28]. Additionally, the growth of cucumber plant was increased of 25 and 40% in the dry weight of roots and shoots, respectively after inoculated with the potential biocontrol agent *T. harzianum*[29].

6. Application of Antagonistic Microorganisms

Baker[30] reported that the application of antagonistic microorganisms of plant diseases control actually developed as an academic field during the 1970s. Nowadays, it is a complete discipline supported in the public area. Research related to the implementation of these microorganisms is published in many scientific journals such as Biological Control, Biological Research and Technology and BioControl[3,6,31].

Over the past forty years, much research in antagonistic microorganisms has conducted. However, researchers need to look forward for new and different approaches to facilitate new plant disease control technologies and applications. Presently, knowledge in various fields such as computer science, mathematics, statistics, molecular biology and chemistry have directed the recent research aimed at illustrating the structure and functions of antagonistic microorganisms, plant pathogens and plant at the molecular, cellular and ecological levels[3,6,32].

At the present, numerous antagonistic microbial based products are being sold for control the plant diseases caused by fungi. An increasing number of companies are also creating products which are being in the process of registration. Many of them are small companies and have a limited product line. Others are widely traded and have significant capitalization cost. Moreover, larger companies with more different product lines that include various biotechnological products have played a major role in the development and the trade of products for the inhibition of the causal agents of plant diseases[33].

Antagonistic microbial products are either marketed as stand-alone products or formulated as combinations with other microorganisms. Some of them with biocontrol properties may not be registered. On the other hand, they are sold as an alternative, as crop strengtheners or growth promoters with no particular argument concerning plant disease control[33]. In addition, Heydari and Pessaraki[6] suggest that to help develop the worldwide market perception of biocontrol agents as an effective product, the biocontrol agents Industry Alliance is establishing a guarantee process to certify industry standards for efficiency, quality and constancy. In order to increase the application of these agents, it is very

significant to emphasize on some factors such as training of farmers, formulation of antagonistic microorganisms and studying the function of environmental factors.

7. Successful antagonistic microorganism used in Thailand

There are a number of plant diseases control researches focusing on a practical use of antagonistic microorganisms in a crop field. The microorganisms such as *Trichoderma* spp., *Streptomyces* spp., and *Bacillus* spp. have successfully been used for plant diseases control worldwide[16,34,35,36]. In Thailand, they have also been studied and applied. Therefore, in the particularly section we focus on some benefits antagonistic microorganisms.

Trichoderma harzianum is one of the most effective antagonistic microorganisms in Thailand. It is able to broadly control many plant pathogens for instance *Rhizoctonia solani*[37], *Sclerotium rolfsii, Pythium* spp.[38,39,40], *Phytophthora* spp.[41], *Fusarium* spp.[42,43] and *Macrophomina phaseolina*[44]. In 1996, *Trichoderma* commercial product named the Unigreen UN-1 was the first product registered in Thailand[45]. Apart from using an instant commercial product, farmers can culture and propagate *Triderderma* on their own. However, they probably have a contamination problem, if they do not have an aseptic skill and do not use an appropriate material and method.

Bacillus spp. are another group of antagonistic microorganism that successfully used. These bacteria have been studied to control a wide spread of many plant pathogens in Thailand. For example, *B. subtilis* can use for control of root and stem rot disease of durian caused by *Phytophthora palmivora*[46] and tomato gray mold disease caused by *Botrytis cinerea*[47]. *Bacillus megaterium* SBK5.7 and *Bacillus* sp. SPT41.1.3 reduced the disease severity of chilli anthracnose on seed[48]. *Bacillus* sp. HT-NK-460 and TZ-CP-342 had 97.22-100% inhibitory effect on spore germination of *Cercospora cruenta*, *Uromyces vignae* and *Oidium* sp.[49].

Streptomyces spp. are a group of Gram positive bacteria that can be used for antagonistic microorganism against many significant plant pathogens such as *S. philanthi* RL-1-178 could as inhibit *Sclerotium* root and stem rot and *Ralstonia* wilt of chili pepper as equal to carboxin and streptomycin sulfate treatment, respectively. Moreover, this antagonistic microorganism could effectively protect chilli plants from *S. rolfsii* and *R. solanacearum* under field conditions[34]. Furthermore, Thummabenjapone and Choksan[50] reported that *Streptomyces*-15, *Streptomyces*-22, *Streptomyces*-84 and *Streptomyces*-87 could inhibit *Acidovorax avenae* subsp. *citrulli* and *Ralstonia solanacearum* by bioassay test.

In conclusion, the antagonistic microorganisms found in Thailand are high efficiency plant disease control agents. Some of them are being examined and have not been reported so far. In case of further benefit, antibiotics produced by these microorganisms are probably useful for animal and human disease treatment.

8. The Advantages and Disadvantages of Diseases Control Using Antagonistic Microorganisms

Regarding the advantages of the application of antagonistic microorganisms, generally, consumers and growers are concerned about their health, safety and the environment. There are much evidence and research that commercial pesticides affect both an organism and the environment. Therefore, the acceptance of the biological plant disease control approaches using antagonistic microorganisms is various promoting in crop production. In case of efficiency and reliability, Fravel[51] states that the most achievements in biocontrol have been accomplished in circumstances where environmental conditions are most controlled or expected and where antagonistic microorganisms can protectively colonize the infection area. In addition, Kessel[52] and Heydari and Pessaraki[6] affirm that soilborne and postharvest diseases have been eliminated successfully by using these microorganisms. They take action as bioprotectants, for instance preventing diseases. Particular applications for the great value crops targeting certain disease such as downy mildew, powdery mildew and some other diseases have also been implemented[6,52]. Consequently, as research solves the numerous situations desired for successful control of diverse diseases, the implementation of antagonistic microorganisms in crop production is bound to expand in the future.

Considering the disadvantages of the application of antagonistic microorganisms, its application in plant diseases control has been slow mainly because of their inconsistent performances under diverse ecological conditions in the crop field. Many antagonistic microorganisms achieve perfectly in the laboratory and green house situations but fail to work in the crop field. This difficulty can only be solved by improved knowledge of the environmental factors that have an effect on them[53,54]. In addition to this difficulty, there has also been rather little investment in the enhancement and creation of trade formulation of these microorganisms because of the budget allocated to invention, examination, registration and promotion of these products[6,33].

Conclusion

In recent year, there has been an increasing use of chemical pesticides. However, there are concerns on their contamination of the environment and their effect on non target organisms. Biological control of plant diseases is an alternative safer approach. This review suggests the application of the plant diseases control using antagonistic microorganisms instead of chemical pesticides including its detail and discussion.

Biological control of plant diseases by using antagonistic microorganisms refers to the inhibition of one plant pathogen by one antagonist in a crop production system. It is highly probable that the disease control mechanisms which include antibiosis, competition and parasitism, induced systemic resistance, hypovirulence and induced growth response work in all natural and managed environments. Complete ejection of the chemical pesticide usage in the agricultural system might be unfeasible; however, a reasonable decrease in their use is certainly practicable.

In order to achieve the agricultural and food safety policy goal, which is the guiding principle of the World Trade Organization (WTO), the Common Agricultural Policy (CAP) and many national governments, a sustainable and an organic agricultural system with lowest contamination level and risks to the environment must be widely introduced. Therefore, the use of antagonistic microorganisms should be employed to manage plant disease problems in place of hazardous pesticides. Implementation of this method could be the safest key to the management of such problems in every crop production system.

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