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CONTRIBUTION OF SUPPRESSIVE SOIL IN CONTROLLING PLANT DISEASES

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Abstract

Some soils have been observed to suppress diseases in crops grown upon them. Soils are a rich source of microbes that are thought to help plants suppress pathogens by improving the health of the plant, induce natural plant defense, produce antibiotics, compete against pathogens, or hyperparasitize the pathogen. Soil that suppresses crop disease due to the specific structure of its microbial community is known as disease-suppressive soil. Suppressive soil is an attractive method of biocontrol, because it has the potential to be sustainable over many seasons under favourable conditions.Suppressive soil is an example where the microflora in the soil is effective in protecting plants against soil-borne pathogens. The diversity and density of populations (bacteria, actinomycetes and fungi) is higher in suppressive soil than in conducive soil, including the diversity and population density of antagonistic microbes, for example in banana plant habitats without symptoms of Fusarium wilt (suppressive soil) is higher than the diversity of soil microbes in banana plant habitat with Fusarium wilt symptoms (conducive soil).

Keywords

Suppressive soil, conducive soil, biocontrol, favourable condition, and microbes

Introduction

Pathogens are very important plant pest agents that can cause damage and loss of agricultural products. Pathogens need to be controlled to ensure certainty of clothing and food production and maintain the quantity and quality of production [1, 2]. Several control techniques have been used to manage plant diseases but they still rely on the use of chemical pesticides [3]. The use of chemical pesticides to control plant pathogens, such as fumigation to control soil-borne diseases, can cause side effects because it can kill useful microbes in the soil [4], pollute the environment and disrupt agroecosystems [5].

Another environmentally friendly control alternative uses microbes in the soil. Higa and Parr (1994) [6] and Singh (2007) [7] classify soil on the basis of microbial function, namely: (1) Conducive soil, namely soil that induces disease, where pathogenic microbes such as *Fusarium* can reach 5 – 20% of the total soil microflora. (2) suppressive soil, namely soil that is usually dominated by antagonistic microbes that produce a number of antibiotics, including fungi (*Penicillium, Trichoderma* and *Aspergillus*), and actinomycetes (genus *Streptomyces*). (3) zymogenic soil, namely soil dominated by microflora which can form useful products from the firming process such as the breakdown of complex organic molecules into simple organic substances and inorganic materials, for example plant residues, manure, green manure and compost. (4) Synthetic soil is soil that contains a microbial population capable of binding atmospheric nitrogen and carbon dioxide into complex molecules such as amino acids, proteins and carbohydrates.

One of the functions of microbes in soil is to control soil-borne pathogens, often referred to as antagonistic microbes, while the mechanism used to reduce pathogen survival or pathogenicity is called antagonism [8]. Antagonistic microbes can be explored from naturally available suppressive soils. In suppressive soil, it is possible that pathogens do not exist, or pathogens exist but are not able to cause disease, or pathogens that exist can cause disease but the disease that occurs is of less economic importance. On the other hand, soil where disease can develop well is called conducive soil [9]. If the total microbial biomass is responsible for suppressing soil-borne pathogens, either through competition for nutrients or living space or often directly through antagonism, then the soil is called "general suppression", whereas if only one type of microbe is responsible it is called "specific suppression." [10, 11].

Suppressive soil has been widely studied to control several plant pathogens such as: *Gaeumannomycesgraminis* var. *tritici* (causes take-all disease) in cereals, *Fusarium oxysporum* (causes vascular wilt disease) [12], Aphanomyces euteiches (causes root rot in peas), *Heteroderaavenae* (parasitic nematode), *H. schachtii* (parasitic nematodes), *Meloidogyne* spp. (root knot nematode), *Criconemellaxenoplax* (ring nematode), Thielaviopsis basicola (causes black root rot disease in potatoes and cabbage), *Phytophthora cinnamomi* (causes root rot disease in annual plants), Phytophthora infestans (causes leaf blight disease in potatoes), *Pythium splendens* (causes root rot in avocados), *Pythium ultimum* (causes damping off disease), *Rhizoctonia solani* (causes root rot in wheat), Streptomyces scabies (causes scab disease in potatoes), *Plasmodiophorabrassicae* (causes clubroot disease in cabbage), and *Ralstonia solanacearum* (bacterial rot of tomatoes) [10].

Population and Types of Antagonist Microbes in Suppressive Soils

Soil microbial diversity is related to the percentage of soil-borne diseases, the higher the soil microbial diversity, the lower and the percentage of disease [13]. For example, the population and type of antagonistic microbes are negatively correlated with the percentage of Fusarium wilt disease in banana plants. The higher the population density and type of antagonistic microbes, the lower the percentage of disease. This is due to the population of *Fusarium oxysporum*f.sp. *cubense* (Foc) is increasingly less present in suppressive soils [14] (Sudarma, 2011). A similar thing was also found where the population of *Fusarium oxysporum* in the rhizosphere of melon plants in suppressive soil was lower than in conducive soil [9, 15].

The diversity index and microbial population density in suppressive soil were higher than in soil conducive to Fusarium wilt. The microbial diversity index in suppressive soil is 2.03 which is higher than conducive soil, namely 1.91 [16]. This proves that the role of diversity and number of soil microbial populations is very important in suppressing Fusarium wilt disease in banana plants.

The population density and number of types of microbes antagonistic to Foc found in suppressive soil were greater than in conducive soil, of all the microbes found in the population of Bacillus sp. highest compared to other antagonistic microbes (0.2 x 10^6cfu/g soil) [14], this is possible because bacteria are able to reproduce faster than actinomycetes and fungi [17].

The results of research by Casale (1990) [18] which analyzed suppressive soil, can suppress the development of Phytophthora root rot disease on avocado plants caused by *Phytophthora cinnamomi*. Antagonistic microbes such as *Trichoderma* spp. often found in suppressive soils. Soil-borne diseases develop due to a decrease in microbial diversity in the soil, the level of ability to suppress disease is related to the total activity of microbes in the soil. Highly active microbes in the soil require carbon, nutrients and energy, so that the diversity and number of competitors, inhibitors and predators becomes higher, which in turn has implications for reducing the presence of pathogens in the soil [19].

Microbial Interactions in Soil

There are several interactions that occur between two or more microbes in soil, namely: mutualism, protocooporation, commensalism, neutralism, antagonism, competition, parasitism, and predation [1, 2, 20].

Mutualism is a relationship between two or more species where both benefit each other. Obligate long-term interactions include physical and biochemical contact relationships, for example between plants and mycorrhizal fungi. Generally, interactions that occur saprophytically are mutually beneficial, such as bacteria of the genus Rhizobium which can reproduce in the soil or have a mutualistic relationship with legume plants. This mutualistic relationship can contribute to biological control, by improving nutrients and increasing host resistance.

Protocooporation is a form of mutualism but the microbes involved are not completely dependent on other microbes to survive. Many microbes isolated and classified as biological control agents can be facultative mutualists, because they rarely survive depending on a particular host, disease suppression is very dependent on initial environmental conditions.

Cammensalism is a symbiotic interaction between two living organisms, where one organism benefits and the other is not affected or does not benefit. Very many microbial relationships with plants are assumed to be commensalism, because their existence individually or in total, is rarely responsible positively or negatively for plants. While its presence may result in various barriers to pathogen infection, the absence of a measurable reduction in pathogen infection or disease intensity is indicative of a cammensalism interaction.

Neutralism is a biological interaction when the population density of one species absolutely does not affect other species. The relationship in biological control is the inability to associate population dynamics of a pathogen with other organisms indicating neutralism.

Antagonism is the interaction of two or more species of organisms that results in negative effects on one or both species of organisms.

Competition is the interaction of two or more species of organisms that results in decreased growth, activity and fertility.

Parasitism is the symbiosis of organisms that are not phylogenetically related to each other over a long period. This type of relationship, one organism benefits, is usually physically smaller than the second (called the parasite), and the other (called the host) is harmed. The activity of various hyperparasites, namely agents that parasitize plant pathogens, can be used in biocontrol. An interesting example is that host infection and parasitism by avirulent pathogens can represent biological control of virulent pathogens by triggering host resistance systems.

Predation refers more to the act of preying on or killing another organism for consumption. The term predator is intended more for animals that eat at a higher trophic level. Predation is also applied to the activities of microbes, protists, and mesofauna, for example fungi feed on nematodes and microarthropods, which consume pathogen biomass.

Biological control can result in different types of interactions, depending on the environmental context in which they occur. Significantly successful biological control, as defined above, most commonly occurs by manipulating mutualisms between microbes and their plant hosts or by manipulating antagonisms between microbes and pathogens.

Mechanism of Inhibition of Antagonist Microbes Against Pathogens in Soil

There are several mechanisms for inhibiting antagonistic microbes against soil borne pathogens, namely: antibiosis, competition, siderophores, mycoparasitism, cell wall breaking enzymes, inducing plant resistance, and plant growth promoting rhizobacteria/PGPR [1, 2, 21].

Antibiosis

Antibiosis plays an important role in suppressing soil-borne diseases caused by certain pathogenic bacteria and fungi. Antibiotics are produced by microbes as a result of interactions with low molecular weights which have a direct effect on other microbes [21; 22; 23]. An example of the antibiotic phenazine (Phz) produced by Pseudomonas fluorescens strain 2-79 which is used to control take-all disease in wheat plants caused by *Gaeumannomycesgraminis* var. *tritici*. The antibiotic agrocin 84, produced by the bacteria *Agrobacterium radiobacter* strain K84, can be used to control tumor disease (grown gall) caused by virulent strains of A. tumefaciens. Trichoderma antagonist fungi can suppress damping off disease in cotton seedlings caused by *Phytium*[24].*Streptomyces* sp. found from suppressive soils are able to suppress Foc through an *in vitro* antibiosis mechanism [25].

The production of antifungal metabolites (enzymes and metal binders) produced by bacteria in vitro can also have activity in vivo. These metabolites include ammonia, butyrolactone, 2,4-

diacetylphloro-glucinol, HCN, kanosamine, Oligomycine A, Oomycine A, phenacin-1-carboxylic acid, pyuluterin, pyrrolnitrin, viscosinamide, xanthobaccin, and zwittermycin[26].

Competition

Based on the perspective of microbes, soil and plant surface life, nutrients are often the limiting environment. Microbes that successfully colonize the rhizoplane must effectively compete for available nutrients. On the plant surface, the host provides nutrients including exudates or decayed tissue. Nutrients can be taken from the waste of other organisms such as insects (for example, aphid honeydew on the surface of plant leaves) and soil. Direct interactions are very difficult to prove, because based on the fact that there is a lot of competition between pathogens and non-pathogens for nutrient sources, this is important to reduce the percentage and intensity of disease. In general, soil-borne pathogens such as Fusarium and Pythium species that infect through contact with mycelium are more susceptible to competition from soil microbes and microbes associated with plants. Pathogens that germinate directly on plant surfaces and are infected via appressoria and infection pegs are more resistant to competition [1].

The production of plant glycoproteins called agglutinins correlates with P. putida colonizing the root system. Mutant *P. putida* bacteria are not capable enough to reduce rhizosphere colonization capacity and reduce Fusarium wilt disease in cucumber plants. The high abundance of non-pathogenic microbes associated with plants generally protects the plant through rapid colonization, thereby limiting the substrate, and making it unavailable for pathogen growth. Instances of nutrient catabolism in the spermosphere were identified as a mechanism contributing to the suppression of *Pythium ultimum* by *Enterobacter cloaceae*. These microbes at the same time produce metabolites that are able to suppress pathogens, colonize places where there is a lot of water and carbon, such as secondary roots, damaged epidermal cells, nectar and use root mucilage [1].

Siderophore

All plants, animals and microbes except Lactobacilli and Borrelia species cannot survive without iron. Most enzymes bind iron in their activity. More than 105 iron ions are required in the key metabolic processes of one bacterial cell. Siderophore is a compound that has the ability to bind Fe3+ ions very strongly, so that pathogens cannot obtain Fe3+ ions for the formation of spore germ tubes. This siderophore formation can be produced by P. fluorescens [3]. Competition between pathogenic bacteria and fungi for space and nutrients has been widely known as a biological control mechanism, now what is interesting to study is competition for iron. Under conditions of iron limitation, bacteria produce iron-binding compounds called siderophores. Many *Psuodomonas*species produce the siderophore pyoverdine which is used to control Pythium and *Fusarium* species [27].

Micro nutrients such as iron are very important. Iron is limited in the rhizosphere depending on soil pH. In soils with high aeration and oxidation, iron is present in the form of Ferric, which is insoluble in water (pH 7.4) and concentrations may reach 10^{-18} M. This concentration is too low to support microbial growth, which generally requires iron at a concentration of 10^{-6} M. Microbes to survive in every environment, secrete siderophores which have the ability to bind iron from the environment. Almost all microbes produce siderophores, either catechol or hydroxamate [1, 24].

Mycoparasitism

The mycoparasitism mechanism is where the pathogen is directly attacked by a biological agent that can kill it. Four main groups of hyperparasites are known, namely: hypoviruses, facultative parasites, obligate pathogenic bacteria and predators. An example of a hypoparasite is a virus that infects *Cruphonectriaparasitica* (the fungus that causes Chestnut blight) which causes hypovirulence which can reduce the pathogenicity of the pathogen. The interaction of viruses, fungi and the environment determines the failure and success of hypovirulence.

Hypoviruses of several hypoparasitic fungi have been known including one that attacks sclerotia (*Coniothyriumminitans*) or another that attacks fungal hyphae (*Pythium oligandrum*). In some cases one pathogenic fungus can be attacked by many hyperparasites. Examples are *Acremonium alternatum, Acrodoniumcrateriforme, Ampelomycesquisqualis, Cladosporium oxysporum* and *Gliocladium virens*. Some biological agents are predatory under conditions of nutrient limitation. For example, *Trichoderma* is an antagonistic fungus that produces enzymes that directly attack the cell walls of pathogenic fungi. Fresh material is used as compost, in conditions like this Trichoderma does not directly attack the plant pathogen (*Rhizoctonia solani*). In the decomposition process the available cellulose decreases and this activates the chitinase gene *Trichoderma* sp. which then produces chitinase to parasitize *R. solani*.

Cell Wall Breaking Enzymes

A variety of microbes secretes and excretes metabolites that can influence the growth and activity of pathogens. Many microbes produce and release lytic enzymes that can hydrolyze a variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose and DNA. Expression and excretion of these enzymes by various microbes can sometimes produce pressure for direct pathogenic activity. The example of *Sclerotium rolfsii* by *Serratia marcescens* is mediated by chitinase expression. The α β-1,3-glucanase enzyme contributes significantly as a biological control for *Lysobacterenzymogenes* strain C3. This enzyme can suppress and break down the cell walls of living microbes. Generally, this enzyme works to decompose plant residues and dead organic material.

Systemic Resistence Induces

Each plant reacts to biotic and abiotic stresses that trigger resistance reactions. Plants react to: (1) Physical stress such as heat and cold, (2) inoculation by pathogenic and nonpathogenic organisms, (3) Chemical molecules of natural and synthetic origin expressed by a resistance reaction called systemic induced resistance (SIR) [26]. Induced resistance in plants against various pathogens is known as systemic acquired resistance (SAR). SIR is mediated by jasmonic acid (JA), while SAR is mediated by the formation of salicylic acid (SA) [1]. SAR may be induced by plants inoculated either with necrogenic or non-pathogenic pathogens or by certain natural events or synthetic chemical compounds. This resistance response includes physical thickness of cell walls through lignification, cellulose deposition, accumulation of low molecular weight antimicrobial substances (phytoalexins), and synthesis of various proteins (chitinase, gluconase, peroxidase and other proteins) associated with pathogenesis.

The resistance system is also stimulated when plants are colonized by plant growth promoting rhizobacteria (PGPR). Some PGPR strains are effective in controlling the disease by inducing systemic resistance. Induced systemic resistance has been widely studied primarily in laboratories and greenhouses, now it has been indicated that microbes induce SAR that can protect plants from pathogen infection under field conditions with treatment of these beneficial microbes.

Chemical compounds that induce plant resistance to pathogens include polyacrylic acid, ethylene, salicylic acid and acetyl salicylic acid, various amino acid derivatives, the herbicide phosphinotricin and harpin produced by *Erwinia amylovora*. A lipopolysaccharide compound with antigenic side bonds produced by *P. fluorescens* strain WCS374 is included as an inducer of systemic resistance in spinach plants to Fusarium wilt disease. The CHAO strain is effective in controlling take-all disease in wheat caused by *G. graminis* var. *tritici* and has been found in the root cortex. This strain can produce metabolites as a result of increased stress for plants when the metabolites are in plant cells. This indicates that stress can induce resistance mechanisms against pathogens.

Plant Growth Promoting Rhizobacteria (PGPR)

The concept of PGPR in relation to biological control has been widely studied, where PGPR increases plant growth indirectly, either through suppressing diseases caused by major pathogens or reducing the effects caused by minor pathogens (microbes that reduce plant growth but without showing symptoms). Very many bacteria fall into the PGPR category. Another alternative is that PGPR can increase plant growth by binding N, dissolving nutrients such as P, stimulating mycorrhizal function, regulating ethylene production in roots, releasing phytohormones, and reducing heavy metal toxicity [26]. The success of bacteria as PGPR is highly dependent on their ability to carry out the colonization process, their ability to survive when inoculating seeds, multiply in the spemosphere (the area surrounding the seed) in response to seed exudates, attach to the root surface, and colonize the developing root system [27] (Figure 1).

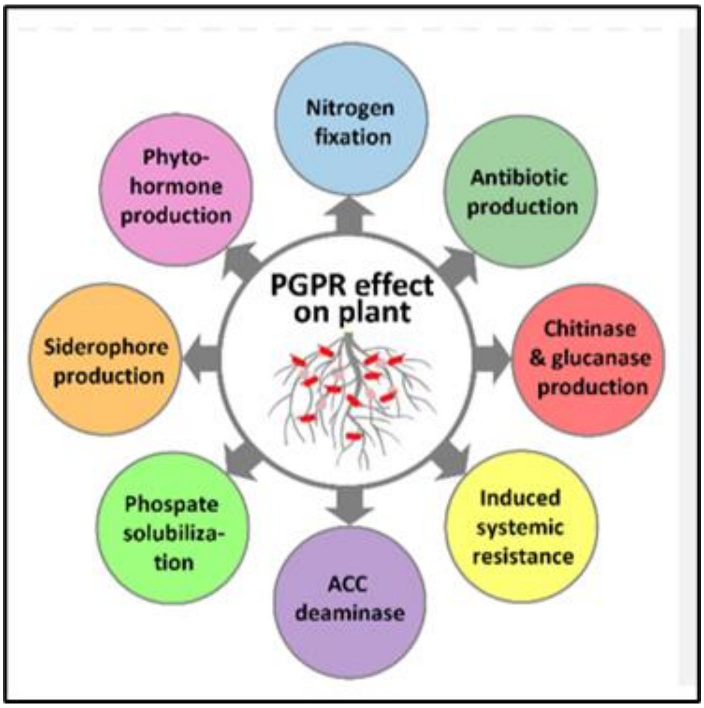

Figure 1. Effect of PGPR on plants [28]

Factors That Contribute to Suppressive Soil

All soils are important if they have the ability to suppress soil-borne diseases. Long-term soil management can reduce or increase the level of disease suppression. A number of management factors are related to the level of soil's ability to suppress disease. This includes intensification of planting, moderate to high nutrient levels, management of weeds, pests and diseases and remaining stubble on the land. All of these factors have the end result of increasing residue returns to the soil, providing a large amount of food for microbial activity. Plants infected by root pathogens, due to loss of thatch and consequently less soil organic matter compared to healthy plants. A lack of hay means food loss and lower microbial activity.

According to Garbeva*et al*. (2004) [13] the ability of soil to suppress soil-borne pathogens is determined by: (1) the type of plant that determines the structure of the microbial community in the soil, such as plants that provide the main carbon and energy sources, (2) the type of soil that determines the structure of the microbial community, such as the combination of structure and texture soil, organic matter, microaggregate stability, pH, and availability of important nutrients such as N, P, and Fe, and (3) Management of agricultural systems such as crop rotation, tillage, herbicide and fertilizer application and irrigation, are also key determinants of community structure microbes in the soil.

The type of plant determines the community structure in the soil, through plant roots which release various compounds into the surrounding soil, including ethylene, sugar, amino acids, organic acids, vitamins, polysaccharides and enzymes. This material creates a unique environment for microbial life in conjunction with plant roots in the rhizosphere [20]. Bacteria respond to differences in compounds released by plant roots, so varying compositions of root exudates are expected to select communities in the rhizosphere. In other words, rhizosphere bacteria will influence plants, such as bacteria with high diversity in the rhizosphere can stimulate plant growth through chemical signals such as auxin, gibberallin, glycolipids and cytokinins. Genera such as *Pseudomonas, Agrobacterium, Bacillus*, *Variovorax*, *Phyllobacterium*, and *Azospirillum* are bacteria that are very efficient at stimulating plant growth. For example, *Azospirillumbrasilense* can have a positive effect on soybean growth and *Agrobacterium tumefaciens* can have a strong effect on plant root development [13]. Soil type determines the structure of the microbial community in the soil, which is based on differences in particle size distribution, pH, cation exchange capacity, and soil organic matter, thereby influencing the structure of the microbial community both directly by providing specific habitats capable of selecting certain microbes and indirectly through influence of root function and plant root exudates [13, 20].

Soesanto (2008) [5] states that the factors that influence biological agents to work optimally in the soil are: crop rotation, soil pH regulation, soil processing, planting time, embedding fresh organic material, regulating irrigation, planting trap crops, manipulating the growing environment, adding biological agents, applying appropriate methods, providing land treatment, and maintaining environmental conditions.

There are several ways to form suppressive soil, namely: (1) crop rotation, besides being able to prevent certain pathogens, it is also useful for improving soil structure and increasing soil organic matter, (2) conservation tillage, which is intended to minimize tillage, at least one third of the soil surface is still covered by plant residue (Stika, 2006), and (3) application of compost and manure which is useful for increasing large amounts of organic matter which can then be degraded by soil microbes and can control infectious pathogens soil [29, 30].

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