ANTIFUNGAL ACTIVITY OF BACTERIA ISOLATED FROM SABAH TEA PLANTATION AGAINST FUNGI ISOLATED FROM DISEASED TEA ROOTS

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

HORTICULTURE AND LANDSCAPING PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2015

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: Antifungal Activity of Bacteria Isolated from Sabah Tea Plantation Against Fungi Isolated from Diseased Tea Roots

IJAZAH: The Degree of Bachelor of Agriculture Science with Honours

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ACKNOWLE DGEMENT

First of all, I would like to express the deepest appreciation to my supervisor, Mr. Clament Chin Fui Seung for the continuous support of my degree study and research, for his patience, motivation, enthusiasm, and immense knowledge. I would like to express my sincere thanks to my co-supervisor, Dr. Mohamadu Boyie Jalloh for his guide and advice that made this research successful. Besides, I would like to appreciate his patience and construction comments during this study.

Moreover, special thanks are extended to all laboratory assistants who always help me in the preparation of apparatus and materials in laboratory works. Thanks also to FSA for providing a comfortable and nice environment that set up with sufficient equipment for the completion of this study.

I would like to thank my parents for their love, support and care for me throughout the period for completion my degree study in UMS and my entire life. Without them, I would not have been able to complete this study. At last, thanks be to God who always grants me His peace, love and wisdom for my study and future.

ABSTRACT

A laboratory experiment was conducted at the Faculty of Sustainable Agriculture in Universiti Malaysia Sabah from 26^{th} June 2014 to 18^{th} October 2014 to study the effect of antifungal activity of bacteria isolated from Sabah Tea Plantation against fungi isolated from disease tea roots. Soil bacteria from Sabah Tea Plantation, Ranau were isolated and investigated their effectiveness against several fungi. Dual culture assay, Nutrient agar amended with bacteria culture test, mycelia growth test and bacteria culture filtrate were carried out to test the antifungal activity of bacteria against several fungi isolated from the disease tea roots. After undergoing preliminary selection, 20 groups of rhizopheric bacteria were successfully obtained. Preliminary screening of these bacterial isolates for antagonistic activity using dual culture assay had been carried out. The effectiveness of the antagonism character were evaluated based on the percentage inhibition radial growth (PIRG) and percentage inhibition diameter growth (PIDG). Among the 20 groups of isolated bacteria, only four bacterial isolates (A1, A2, A4 and A14) showed potential antagonistic activity toward fungi (GD, F4, F5, and F9). They were significant effect on the interaction between bacteria and fungi in Nutrient agar amended with bacteria culture test $\{F\ (9, 48) = 33.835, p<0.05\}$ and mycelial growth test $\{F\ (9, 48) = 45.585, p<0.05\}$. Bacterial isolate A1 and A2 even showed 100% PIDG toward fungi F4 in Nutrient agar amended with bacteria culture test and bacterial A14 had 94.67% PIDG toward fungi F4 in mycelial growth test. These bacteria were then identified with Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and the isolate A1 was *Bacillus cereus*, A2 was *Pseudomonas aeroginosa*, A4 was *Streptomyces grises* and A14 was *Serratia marcescens*.

Kesan Anti-kulat Bakteria Tanah daripada Perladangan Sabah Teh Terhadap Kulat yang Dipemecilkan daripada Penyakit Akar Teh

ABSTRAK

Satu eksperimen makmal telah dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah pada 26^{hb} Jun 2014 hingga 18^{hb} Oktober untuk menguji kesan anti-kulat bakteria tanah daripada Perladangan Sabah Teh terhadap kulat yang dipemecilkan daripada penyakit akar teh. Bakteria tanah dari Perladanagn Sabah Teh, Ranau telah dipemecilkan dan diperiksa keberkesanannya terhadap beberapa kulat. Dual kultur assay, Nutrien agar dipinda dengan kultur bakteria, pertumbuhan mycelia dan kultur bakteria turasan telah dijalankan untuk menguji bakteria terpencil terhadap beberapa kulat yang telah diasingkan daripada akar teh penyakit. Selepas menjalani pemilihan awal, 20 kumpulan bakteria tanah telah berjaya diperolehi. Pemeriksaan awal bagi bakteria kultur untuk aktiviti antagonitik telah dijalankan dengan dual kultur assay. Keberkesanan permusuhan telah dinilai berdasarkan pertumbuhan berdasarkan pertumbuhan jejari peratus perencatan dan pertumbuhan diameter peratusan perencatan. Antara 20 kumpulan bakteria yang dipencil, hanya empat bakteria (A1, A2, A4 dan A14) menunjukkan potensi aktiviti terhadap kulat (GD, F4, F5, dan F9). Interaksi antara bakteria dan kulat adalah signifikasi dalam Nutrien agar dipinda dengan kultur bakteria $\{F (9, 48) = 33,835, p < 0.05\}$ dan ujian pertumbuhan mycelial $\{F (9, 48) = 45,585, p < 0.05\}$. Bakteria A1 dan A2 mewujudkan 100% anti-kulat aktiviti terhadap kulat F4 di Nutrien agar dipinda dengan kultur bakteria and bakteria A14 mewujudkan 94.67% anti-kulat aktiviti terhadap kulat F4 di ujian pertumbuhan mycelia. Bakteria ini telah dikenal pasti dengan MALDI-TOF MS dan A1 adalah *Bacillus cereus*, A2 adalah *Pseudomonas aeroginosa*, A4 adalah *Streptomyces grises* dan A14 adalah *Serratia marcescens*.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

°C	Celsius
%	Percentage
µL	microliter
cm	centimeter
CRD	Complete Randomized Design
EtOh	Ethanol
mins	minutes
mL	milliliter
Na	Nutrient agar
PDA	Potato Dextrore Agar
PGRP	Plant Growth Promoting Rhizobacteria
PIDG	Percentage inhibition of diameter growth
PIRG	Percentage inhibition of radial growth
SPSS	Statistical Package for the Social Science

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3.1 The PIRG 21

$$PIRG = \frac{(R1 - R2)}{R1} \times 100\%$$

R1 = radial growth of test fungus on control plate

R2 = radial growth of fungus towards the antagonist bacteria colony

3.2 The PIDG 21

$$PIDG = \frac{(D1 - D2)}{D1} \times 100\%$$

D1 = diameter growth of test fungus on control plate

D2 = diameter growth of fungus towards the antagonist bacteria colony

CHAPTER 1

INTRODUCTION

1.1 Introduction

Soils sustain a wide variety of prokaryotic and eukaryotic organisms. Some microorganisms are able to colonize the rhizosphere, multiply and colonize plant roots at all stages of plant growth (Kloepper *et al.,* 1980). The bacteria that colonize at the rhizosphere are known as rhizobacteria. One of the most useful bacteria, plant growth-promoting rhizobacteria (PGRPs) are a group of rhizobacteria that metabolite various organic compound from root exudates and exhibit useful effects on plants. In fact, soil usually reflects the appropriate balance between the functions of the microbial community. A healthy soil not only provides essential nutrients that required for plant growth but also provides a good biotic environment for plant to resist against pathogen.

Generally, plant-bacterial interactions can be classified as pathogenic, saprophytic, and beneficial (Lynch, 1990). Beneficial interactions involving PGPRs are important in agriculture as they help to improve plant productivity, suppress disease-causing microbes and nematodes, accelerate nutrient availability and assimilation, and bioaccumulation or microbial leaching of inorganics. These bacteria belong to the genera, *Acinetobacter, Agrobacterium, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, Rhizobium, Serratia*, and *Thiobacillus* (Babalola, 2010). Among of these bacteria, *Bacillus* and *Pseudomonas* species are the major group bacteria and they are widely found in many crop plants.

There are many available reports demonstrated that bacteria as biocontrol agent to suppress the growth of fungal pathogens. For instance, the species of *Pseudomonas* had been reported to produce antifungal metabolites that against the plant deleterious fungi, namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Fusarium oxysporum* and *Sclerotium rolfsii* (Manwar *et al.*, 2004). Microbial plant pathogens may be present in different natural habitats including plants, soil, water, and air. These pathogens may be able to infect numerous plant species or restricted to one or a few host species as obligate parasite or saprophytic parasitic for their nutrition and development during their entire life cycle. It is well known that a significant number of fungal pathogens can survive in the soil and infect the plants, when seeds are sown or young seedlings are planted in the pathogen-infested soil (Narayanasamy, 2013).

PGPRs produce various compounds such as hydrogen cyanide (HCN), phenazines, pyrrolnitrin, pyoluteorin, enzyme, antibiotics, metabolites, and phytohormones that are toxic to pathogens. These PGPRs can suppress disease from one or more mechanisms, including competition, production of antibiotics or siderophores, induction of systemic resistance and secretion of cell wall degrading enzymes and alternation of the plant hormone levels. It has been found out, combining multiple PGPR types can suppress disease development in many crop plants and protect them against a broad range of soil-borne plant pathogens (Singh *et al.*, 2011).

Biological control is a strategy that used to suppress disease and it was proposed half a century ago. The four strategies that common applied in biological control are conservation, classical, augmentative, and importation of biological control. According to Bentely *et al.* (1997) , conservation of biocontrol is the application of natural enemies occurring at a particular site while classical is the introduction of exotic enemies to a new locale where they did not originate or do not occur naturally. Furthermore, augmentative is the supplemental release of natural enemies at critical time and importation biological control is a cost-effective alternative to chemical control for basis food crops.

1.2 Justification of Study

The negative environmental impact of chemical fertilizers and their increasing costs had led to the alternative methods of plant protection, which is the use of beneficial soil microorganisms such as PGPR for sustainable and safe agriculture. PGPR are the microorganisms, which play a major role in the biocontrol of plant pathogens. These PGPRs are widely reported in influencing plant growth, yield, and nutrient uptake by one or combination of several mechanisms. They help in improving the nutrient uptake and enhance the population of beneficial microorganisms in suppressing soil-borne pathogens. According to Cook (1993), microorganisms isolated from the rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species. As early as in 1920, it was noticed that pathogens are suppressed if some antibiotic producing microbes are added to the soil through bioaugmentation (Johri, 2009). The research has also found that microorganisms grow in the rhizosphere are ideal for use as biocontrol agent, since the rhizosphere provides the front-line defense for roots against attack of pathogens. The application of PGPR in agriculture has become a common practice in many regions of the world and there is now an increasing number of PGPR being commercialized as biocontrol agents for various crops (Jayakumar *et al.,* 2002; Saharan and Nehra, 2011). It is believed that, greater application of PGPR is possible for sustainable agriculture in near future which protect the environment, human communities, and animal welfare.

1.3 Objectives

The objectives of this study are:

1. To isolate, purify and identify soil bacteria with antagonistic activity from Sabah Tea Plantation

2. To evaluate the antagonist effect of selected bacteria toward fungi

1.4 Hypothesis

Hypothesis

H_o: There is no significant effect of screening bacteria toward fungi isolated from disease tea roots.

H_1: There is a significant effect of screening bacteria toward fungi isolated from disease tea roots.

CHAPTER 2

LITERATURE REVIEW

2.1 Rhizosphere and Rhizospheric Bacteria

Soil is the habitat of most microorganisms. It acts as a storehouse of microbial activity and aggregates with accumulated organic matter. It also provides foods and essential nutrients for plant growth and development. Rhizosphere is the zone of soil that is influenced by root secretions and it can contain up to 10^{11} microbial cells per gram root and more than 30,000 prokaryotic species (Egamberdieva *et al.*, 2008). Hence, microbial activities under this region are considerable importance to plant health and soil fertility. A significant amount of the organic material photosynthesized in the aerial organs of the plant is released by the living roots in soil. This process is known as rhizodeposition. The organic matter so deposited, comprises of free cap cells, polysaccharides, soluble secretions, lysates and some inorganic nutrients (Whips, 1990). Release of such materials induces change in physic-chemical characteristics of the soils, such as acidity, moisture, electrical conductivity, redox potential and oxygen availability (Lynch, 1990).

In fact, microbial growth is affected by the availability of carbon in the soil. Rhizodeposition makes more available carbon for microorganisms give rhizhosphere an environment with a high microbial diversity. The abundance of microbial activity is usually higher in the upper layer and decrease gradually in the deeper layer. According to a research, the number of microorganisms is higher at root tip compared to other locations along the root (Scott *et al.,* 1995). The distribution and population structure of microorganisms in rhizosphere various for different plants due to the variation in the quantity and quality of the compounds exuded by the plant roots. Thus, the rhizosphere microbial communities also differ with the plant species and there is a wide variety of microorganism can be found in the soil. Among of the microorganisms, bacteria are the abundantly microbes found in the soil.

2.1.1 Rhizobacteria

Rhizobacteria which also known as plant growth promoting rhizobacteria (PGPR) are a group of root-colonizing bacteria in the rhizosphere that metabolize various organic compound from root exudates and exhibit useful effects on plants. PGPR are involved in the biogeochemical cycle and supply nutrient for crops, plant growth stimulation and biological control of plant pathogens. During the past several decades, many researchers have studied various PGPR strains for their capacity to increase the plant growth, yield, and to control the plant pathogens including plant pathogenic fungal and nematodes under greenhouse and field conditions (Jayakumar *et al.,* 2002; Shouan *et al.,* 2010). However, the effects of PGPRs depend mostly on the genotype of the microorganisms and plants involved as well as on the environmental conditions (Brimecombe *et al.,* 2007).

Two group of PGRR were described and the first group is involved in the nutrient cycling and plant growth stimulation while the second group is involved in the biological control of plant pathogens (Whipps, 2001; Vessy, 2003). There are many mechanisms have been proposed for growth promotion by PGRP strains. First, enhancement of plant growth by PGRP was elucidated by the production of compounds that mimic plant hormones or other plant stimulants (Ryu *et al.*, 2003). Second, mechanisms that decrease microbial populations of pathogenic or deleterious microorganisms through antibiosis.

Among of the soil bacteria, *Pseudomonas* spp. and *Bacillus* spp. belong to the largest groups of rhizobacteria and they are able to produce antibiotics or siderophore that can suppress the growth of pathogens. A report of El-Nagdi and Youssef (2004) had showed the rhizobacteria produce antibiotics that directly or indirectly induce resistance and hence reduce nematodes population in the soil.

2.1.2 Endophytic Bacteria

Endophytic bacteria are those bacteria which reside inside the living tissue without doing substantive harm or gaining benefit other than securing residency (Azevedo *et al.,*2000; Surette *et al.,*2003) It has been found that, the endophytic bacteria enhances the plant growth and reduces the disease severity caused by variety plant pathogen (Shaukat *et al.,* 2002).

Same as rhizobacteria, endophytic bacteria also can promote plant growth and yield involved in the inhibition of phytopathogens. They may play a role in soil fertility through phosphate solubilization and nitrogen fixation. Besides, they can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry (Ryan *et al.,* 2008). Endophytic bacteria also have the potential to remove soil contaminants by enhancing phytoremediation.

The common bacterial species of endophytes reside in the plants are *Pseudomonas fluorescens, Bacillus spp.*, *Herbaspirillum* spp., *Serratia marcescens* and *Streptomyces* spp. (McLnroy and Kloepper. 1995). The study also proved that in most plant the population densities of the endophytes are greatest in the plant roots with densities ranging from 10^4 to 10^6 colony forming unit (cfu) per gram fresh weight in cotton and sweet corn roots.

Moreover, bacterial endophytes which act as bio-control agents can be divided into two groups which are extensive colonizers and primarily colonizers. Extensive colonizers of the internal plant tissues suppress the pathogens by niche occupation, antibiosis, or both while primarily colonizers occupy the root where they elicit plant defense or resistances mechanisms. Recently, there is increasing interest in developing the potential biotechnological applications of endophytes for improving phytoremediation and the sustainable production of nonfood crops for biomass and biofuel production (Karp and Shield, 2008)

2.1.3 Parasitic Bacteria

Parasitic bacteria are defined as the microbes which obtain foods and nutrients from the hosts. They are pathogenic which may cause various diseases in plants and animals. However, it also can be a biological control agent that suppresses the population of nematodes in the soil. There are several researches were done to study the biological control of plant-parasitic nematodes and one of the examples of parasitic bacteria that used is *Pasteuria penetrans* (Pandey *et al.* ,2000; Bird *et al.*, 2003).

P.penetrans is an obligate nematode parasitic endospore forming bacteria which widely used to control different genera of plant parasitic nematodes on a various crops. It produces non motile endospores which attach with the cuticle of moving nematode in soil (Davies, 2005). However, the efficacy and implementation of *P. penetrans* was affected by environmental factors such as soil types, texture, soil moisture, and temperature.

2.2 Antagonistic Microorganisms

2.2.1 Gram negative bacteria

Some important groups of PGPR among Gram negative bacteria are belongs to genera *Pseudomonas* and *Burkholderia* (Brahim, 2013).

a) *Pseudomonas*

Pseudomonas is a Gram negative, aerobic and rods shaped bacteria. They are found abundant in the rhizosphere and their ability to suppress the fungal and bacterial pathogens in a wide range of crops have been demonstrate. It is the most important group of PGRP among Gram negative bacteria. The PGPR effect of *Pseudomonas* was largely reported reviewed and it has been recognized as a major PGPR for many crop such as potato (*Solanum tuberosum* L.), radish (*Raphanus sativus* L.), sugar beet (*Beta vulgaris* L.) and lettuce (*Lactuca sativa*) (Lemanceau, 1992).

Within the genus *Pseudomonas, Pseudomonas fluorescens* are ubiquitous bacteria that are most studied group (Weller, 1988). Strains of *P. fluorescens* used in biocontrol have contributed greatly in disease suppression. They are able to compete aggressively for sites in the rhizosphere and prevent proliferation of phytopathogens production of antibiotics and sidespores, or inducing systemic resistance; by stimulating plant growth by facilitating either uptake of nutrients from soil; or by producing certain plant growth promoting substances (De Weger *et al.,* 1986; Haas and Defago, 2005; Spaepen *et al.,* 2007). According to Rezzonica *et al.* (2007), biocontrol strains from *P. fluorescens* or related species may vary from one another in terms of their mode of action, as well as their efficacy at protecting plants.

b) *Burkholderia*

The genus *Burkholderia* contains over 30 species, which occupy remarkably diverse ecological niches, ranging from contaminated soils to the respiratory tract of humans (Tom and Peter, 2003). *Burkholderia cepacia* as a plant pathogen was first reported by

Burkholder in 1950 isolated from putrefactive onions (Allan *et al.,* 2003). This bacterial was later found to be a ubiquitous soil bacterial species and has emerged as an important biocontrol agent of plant pathogens.

B. cepacia is a bacterial complex with 10 genomic species which have similar phenotypical traits and different genetics ones and are called genomovars (Cardona *et al.,* 2005; Chiarini *et al.,* 2006). Strains of *B. cepacia* have been used in biological control of plant diseases and bioremediation in many cases, while some strains are plant pathogens or opportunistic pathogens of humans with cystic fibrosis (Sarangthem *et al.,* 2012).

Several *B.cepacia* species are considered to be beneficial in nature and are widespread in nature particularly in plant environment. Different strains of *B. cepacia* were showed to be effective against *Pythium* damping off and *Aphanomyces* root rot disease of peas (Parke *et al.,* 1991). In addition, Santos *et al.* (2012) reported *B. cepacia* as a promising biological control agent against the causal agent of anthracnose (*Colletotrichum gloeosporioides*), through the production of hydroxamate siderophore. Zaiton *et al.* (2008) established *B.cepacia strain* UPM B3, isolated from symptomless palms and its potential as a biocontrol agent against *Ganoderma boninense*.

2.2.2 Gram positive bacteria

Some important group of PGPR among Gram negative bacteria are *Bacillus*, *Paenibacillus*, *Actinomycetes*, and *Serratia* (Brahim, 2013).

a) *Bacillus*

In the opposite to *Pseudomonas* and other nonspore-forming bacteria, *Bacillus* spp. are able to produce spores which are extremely resistant dormancy forms capable to withstand high temperature, unfavorable pH, and lack of nutrient or water. They are present in an extremely large palette of environment ranging from sea water to soil, and are even found in extreme environment like hot springs (Hoch *et al.*, 1993).

Mechanisms involved in *Bacillus* eliciting plant growth promotion include auxin production, increased uptake available of phosphorus, biocontrol abilities, and induction of systemic resistance (Asaka and Shoda, 1996; Zehder *et al.,* 2000). *Bacillus* strains could repress soil- borne pathogens and induce plant resistance to disease following root colonization. It has been found that, *Bacillus* is the first successful biocontrol agent used against insects and pathogens (Powell and Jutsum, 1993).

Commercial strains of *Bacillus subtilis* have been marked as biocontrol agents for managing fungal diseases of crops (Emmert and Handelsmen, 1999). It is a beneficial bacterium which can promote plant growth by secreting cytokinins and auxins and protect crops against fungal pathogen attack. Despite its spore forming ability, *B. subtilis* also possess several characteristics that enhance its survival in the rhizosphere and thus its effectiveness as a biopesticide (Rosas-Garcia, 2009).

Members of *Bacillus* were reported as producers of antibiotics inhibiting various fungal pathogens including *Fusarium oxysporum* and *Rhizoctonia solani* (Asaka and Shoda, 1996). For instance, it produces a variety of antibacterial agents, including a broad spectrum of lipopetides such as surfactin that are potent biosurfactans (Peypoux *et al.*, 1999).

b) *Paenibacillus*

The genus *Paenibacillus,* earlier included under the genus *Bacillus* are facultatively aerobic, endospore-forming, and Gram positive bacilli. Different species of *Paenibacillus* can induce plant growth by fixing atmospheric nitrogen and producing auxins and cytokinin (Lebuhn *et al.,* 1997; Timmusk *et al.,* 1999).

Several strains of *Paenibacillus polymyxa* have been reported to effectively suppress the development of plant diseases such as seedling blight, wilt and root rot diseases of cucumber and watermelon caused by *F. oyysporum* and *Pythium* spp. (Yang *et al.,* 2004). Beneficial effects were also reported in lodgepole pine (*Pinus contorta*) and spruce (*Picea* sp.) after inoculation of *P. polymyxa* (Shishido *et al.,* 1995).

It has been reported *P. polymyxa* produces peptide antibiotics more commonly and polymyxin is the main type of peptide antibiotics produced by some strains of *P. polymyxa* while other strains secreted different peptides including polypeptins (Ryu *et al.,* 2006). Polymyxin alters cytoplasmic membrane permeability by binding to a negatively charged site in the lipopolysaccharide layer and have bactericidal effect on Gram negative bacteria, especially *Pseudomonas* (Wiese *et al.,* 1998).

In addition of antibiotics, *P. polymyxa* are also able to produce hydrolytic enzyme that play an important role in the biocontrol of plant disease. The finding of Weid *et al.* (2005) stated that, strains of *Paenibacillus brasilensis* produced antifungal compounds which inhibited the mycelial growth of *Rhizoctonia solani* and *Verticilium dahlia* that capable of infecting a wide range of crops.

c) Actinomycetes

Actinomycetes are generally saprophytic soil bacteria, spending majority of their life cycles as spores. They are for the most aerobic bacteria but some of them can also grow in anaerobic environment. Actinomycetes are the origin of numerous antibacterial and antifungal based compounds. They represent a high proportion of the soil microbial biomass and able to produce a wide range of antibiotics and extracellular enzymes.

The genus Streptomyces was proposed by Waksman and Henrici for aerobic and spore forming actinomycetes (Williams *et al.,* 1989). Streptomyces are Gram- positive bacteria with a filamentous form similar to fungi. Merriman *et al.* (1974) reported the use of the *Streptomyces griseus* isolate with biocontrol abilities toward *R. solani* in carrot. In another study, one *Streptomyces* isolate genetically close to *Streptomyces kasugaensis* able to inhibit the growth of *Fusarium* and *Armillaria* pine rot (de Vasconcellos and Cardoso 2009). Recently, some reports demonstrated that plant promotion relies on the ability of actinomycetes to solubilize phosphate or to produce phytohormones (El-Tarabity *et al.*, 2008; Hamdali *et al.*, 2008).

In fact, rhizosphere competence of the biocontrol agents is a critical requisites for effective suppression of soilborne pathogen. *Steptomyces griseoviridos* has been reported as a good rhizosphere colonizer and it was found to be an efficient antagonist of fungal pathogens, causing diseases such as *Fusarium* wilt disease of carnation, damping off disease of brassica and root rot disease of cucumber (Tahvonen and Lahdenpera, 1988).

d) *Serratia*

The genus *Serratia* includes *Serratia liquefaciens*, *Serratia marcescens*, *Serratia plymuthica*, and *Serratia rubidaea*. The *S. plymuthica* as endophyte of plants, has been found in the rhizosphere and demonstrated to be a biocontrol agent that can suppress the disease caused by many soil borne pathogens. The strains of *S. plymuthica* showed antifungal activity against *Verticilium dahlia, Rhizoctonia solani* and *Sclerotinia sclerotiorum* in vitro bioassay (Kurze *et al.,* 2001)

S. marcescens is rhizobacterial specie that capable of inducing systemic resistance in plants against fungal and bacterial plant pathogens. It has been found the strain elicited induced systemic resistance (ISR) in cucumber against *Pseudomonas syringae* pv. *lachrymans, Colletotrichum orbiculare* and *Erwinia tracheiphila* under field conditions (Press *et al.,* 1997). Another study showed that, culture filtrate of *S. marcescens* exhibited chitinase and protease activities significantly suppressed the development of damping off disease in cucumber (Robert *et al.,* 2007).

Moreover, the strains of *Serratia* as biocontrol agents parasitic on fungal pathogens excrete extracellular cell wall-degrading enzymes such as chitinases, glucanases, and proteases that target pathogen cell wall, resulted in lysis of the pathogen cells. The effectiveness of *S. marcescens* as a biocontrol agent against *Phytophthora parasitica* causing gummosis disease was reported by de Queiroz and de Melo (2006). The *S. marcescens* lysed the oospores of *P. parasitica* and the application had reduced the citrus seedling infection by 50%.

2.2.3 Fungi

For fungi, the most important PGPR is *Trichoderma.*

a) *Trichoderma*

Several researched had proven that, *Trichoderma* spp. are natural boon to farmers and this microbial antagonists commonly isolated from the place of decaying organic matter, arable soil, orchards and plantation fields (Harman and Kubicek, 1998; Pandey *et al.,* 2000).

In fact, *Trichoderma* is a fast growing fungus, secondary opportunistic invader, strong spore producer and antibiotic producer and a source of cell wall degrading enzymes. It is a fungus that can be found in all climatic zones. It enhances the plant growth, increases the nutrient uptake and induces resistance against plant pathogens. Moreover, *Trichoderma* spp. also increases the production of PR proteins which helps to accumulate more phytoalexin and hence, directly induced the resistance against plant pathogens.

REFERENCES

Adams, P. B. and Fravel, D. R. 1993. Dynamics of Sporidesmium: A Naturally Occurring Fungal Mycoparasite. In Lumsden, R. D., and Vaughn, J. L. (Eds.). *Pest Management: Biologically based Technologies.* Washington: American Chemical Society

Ademir, S. F., Luiz, F. C., Valdinar, B. S., and Romero, F. V. 2009. Soil Microbial Activity in Conventional and Organic Agricultural System. *Sustainability* **1**: 268-276

Adriana, C., Giovanna, P., Sara, M., Mirko, B., Chiara, G., Sabina, R., Maria, C. A. and Flora, D. 2012. MALDI-TOF MS Analysis of Human and Animal *Brachyspira* Species and Benefits of Database Extension. *Journal of Proteomics* **78**: 273-280

Adriana, C., Giovanna, P., Sara, M., Mirko, B., Chiara, G., Sabina, R., Maddalene, P., Flora, D., Maria, C. A., Carlo, C. and Maria C. M. 2014. Leptospira Species and Serovars Identified by MALDI-TOF Mass Spectrometry after Database Implementation. *Research Note* **7**: 330

Ahmadzadeh, M., Afharmanesh, H., Javan-Nikkah, A., Sharifi-Tehrani. 2006. Identification of Some Molecular Traits in *Fluorescent pseudomonas* with Antifungal Activity. *Iranian Journal of Biotechonology* **4**: 245-253

Ahmad, F., Ahmad, I. and Khan, M. S. 2008. Screening of Free-living Rhizospheric Bacteria for Their Multiple Plant Growth Promoting Activities. *Microbiology Result* **163**:173–181

Ahmed Idris, Labuschagne, H., and Korsten, L. 2007. Screening Rhizobacteria for Biological Control of *Fusarium* Root and Crown Rot of Sorghum in Ethiopia. *Biological Control* **40**: 97–106

Allan, N. D., Kooi, C., Sokol, P. A. and Beveridge, T. J. 2003. Putative Virulence Factors are released in Association with Membrane Vesicles from *Burkholderia cepacia. Canadian Journal of Microbiology* **49**: 613-624

Anita, P., Shipra, S. and Lok, M. S. 2013. Microbial Inoculant to Support Tea Industry in India. *Indian Journal of Biotechnology* **12**: 13-19

Asaka, O. and Shoda, M. 1996 Biocontrol of *Rhizoctonia solani* Damping-off of Tomato with *Bacillus subtillis* RB14. *Applied and Environmental Microbiology* **62**: 4081–4085

Azevedo, J. L., Maccheroni, W. J., Pereira, J. O. and Luiz de Araujo W. 2000. Endophytic Microorganisms: Review on Insect Control and Recent Advances on Tropical Plants. *Electronic Journal of Biotechnology* **3**: 40-65

Babalola, O. O. 2010. Beneficial Bacteria of Agricultural Importance. *Biotechnology Letters* **32**: 1559-1570

Bentely, J. W. and Neil, R. J. 1997. On the Ethics of Biological Control of Insect Pests. *Agriculture and Human Values* **14**: 283-289

Bentley, S.D., Chater, K.F. and Cerden, A. M. 2002. Complete Genome Sequence of the Model Actinomycete Streptomyces coelicolor. *Nature* **417**:141–7.

Bird, D. M., Opperman, C. H. and Davies, K. G. 2003. Interaction between Bacteria and Plant-parasitic Nematodes: Now and then. *International Journal for Parasitology* **33**: 1269-1276

Brahim, B. 2013. *Bacteria for Plant Growth Promotion and Disease Management.* Berlin: Springer

Brimecombe, M. J., Leij, D and Lynch, J. M. 2007. *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface.* New York: CRC Press

Cardona, S. T., Wopperer, J., Eberl, L. and Valvano, M. A. 2005. Diverse Pathogenecity of *Burkholderia cepacia* Complex strains in the Caenorhabditis elegans Host

Model. *Federation of European Microbiological Societies Microbiology Review* **14**: 277-286

Chandra, S. N. and Patrice, D. 2008. *Molecular Mechanisms of Plant and Microbe Coexistence*. Berlin: Springer

Chet, I. and Baker, R. 1980. Induction of Suppressiveness to Rhizoctonia solani in Soil. *Phytopathology* **70**: 994-998

Chet, I. and Baker, R. 1981. Isolation and Biocontrol Potential of *Trichoderma harmatum* from Soil Naturally Suppressive to *Rhozoctonia solani*. *Phytopathology* **71**: 286-290

Chet, I. 1987. *Innovative Approaches to Plant Disease Control*. New York: John Wiley & Sons Publishing

Chiarini, L., Bevivino, A., Dalmastri, C., Tabacchioni, S. and Visca, P. 2006. *Burkholderia cepacia* complex species: Health Hazards and Biotechnological Potential. *Trends in Microbiology Journal* **14**: 277-286

Chong, K. P. and Noor Fazila. 2012. Potential Antagonist Organisms against *Poria hypolateritia* of Red Root Disease in Tea Plantation. *International Journal of Agriculture and Biology* **14(3)**: 457-460

Clara, P., Caya, R., Antonio, D. V. and Francisco, M. 2010. Screening for Candidate Bacteria Biocontro Agents Against Soil-borne Fungal Plant Pathogens. *Plant Soil* **340**: 505-520

Cook, R. and Baker, K. F. 1983. *The Nature and Practice of Biological Control of Plant Pathogen*. United State: American Phyto-pathological Society

Cook, R. J. 1993. Making Greater Use of Introduced Microorganisms for Biological Control of Plant Pathogens. *Annual Review of Phytopathology* **31**: 53-80

Davies, K. G. 2005. Interaction between Nematodes and Microorganisms: Bridging Ecological and Molecular Approaches. *Advances in Applied Microbiology* **57**: 53-78

De Queiroz, M. and de Melo. Y. 2006. Antagonism of *Serratia marcescens* toward *Phytophthora parasitica* and its Effects in Promoting the Growth of Citrus. *Brazilian Journal of Microbiology* **37**: 448- 450

De Vasconcellos, R. and Cardoso, E. 2009. Rhizospheric Streptomycetes as Potential Biocontrol Agents of Fusarium and Armillaria Pine Rot and as PGPR for *Pinus taeda*. *Biocontrol* **54**: 807–816

De Weger, L., Van, B. R., Van Der, B. B., Gruters, R. A., Geels, F. P. and Lugtenberg, B. 1986. Siderophores and Outer Membrane Proteins of Antagonistic, Plant-Growth-Stimulating, Root Colonizing *Pseudomonas* spp. *Journal of Bacteriology* **165**: 585–594

Dikin, A., K. and Seman, I. A. 2006. Antagonistic Bacteria Against *Schizophyllum commune* in Peninsular Malaysia. *The Southeast Asian Journal of Tropical Biology* **13:** 111-121

Edkona, J. K., Wong, S. K., Stephen, L. C. T., Make Jiwan, Zakry Fitri Abdul Aziz, Franklin., Osumanu, H. A. and Nik Muhammad Abdul Majid. 2013. Antagonistix Activities of Endophytic Bacteria against Fusarium Wilt of Black Pepper (*Piper nigrum*) *International Journal of Agriculture and Biology* **15(2)**: 2 291-296

Egamberdieva, D., Kamilova, F., Validov, S., Gafurova, L., Kucharova, Z. and Lugtenberg, B. 2008. High Incidence of Plant Growth Stimulating Bacteria Associated with the Rhizosphere of Wheat Grown on Salinated Soil in Uzbekistan. *Environmental Microbiology* **10**:1-9

El-Nagdi, W. M. and Youssef M. A. 2004. Soaking Faba Bean Seed in some Bioagents as Prophylactic Treatment for Controlling *Meloidogyne incognita* Root-Knot Nematode Infection. *Journal of Pest Science* **77**: 75-78

El-Tarability, K. A., Soliman, M. H., Nassar, A. H., Al-Hassani, H. A., Sivasithamparam, K., McKenna, F. and Saint, H. 2000. Biological Control of *Sclerotium minor* using a Chitinolytic Bacterium and Actinomycetes. *Plant Pathology* **49**: 573-583

El-Tarabily, K. A., Nassar, A. H. and Sivasithamparam, K. 2008. Promotion of Growth of Bean (*Phaseolus vulgaris* L.) in a Calcareous Soil by a Phosphate-Solubilizing, Rhizosphere- Competent Isolate of *Micromonospora endolithica*. *Applied Soil Ecology* **39**:161–171

Emmert, B. A. E, Klimowicz, K. A., Thomas, G. M. and Handelsman, J. 2004. Genetics of zwittermicin: A production by *Bacillus cereus*. *Applied Environment Microbiology* **70**: 104-113

Emmert, B. A. E. and Handelsman, J. 1999. Biocontrol of Plant Disease: A Gram Positive Perspective. *Federation of European Microbiological Societies Microbiology Microbiology Letter* **171**: 1-9

Errakhi, R., Bouteau, F., Lebrihi, A. and Barakate, M. 2007. Evidences of Biological Control Capacities of *Streptomyces* spp. Against *Sclerotium rolfsii* Responsible for Damping-off Disease in Sugar Beet (*Beta vulgaris*). *World Journal Microbiology Biotechnology* **23**:1503-1509

Farah Ahmad, Iqbal Ahmad and Khan, M. S. 2008. Screening of Free-living Rhizospheric Bacteria for their Multiple Plant Growth Promoting Activities. *Microbiological Research* **163**: 173- 181

Fox, A. 2006. Mass Spectrometry for Species or Strain Identification after Culture or Without Culture: Past, Present, and Future. *Journal of Clinical Microbiology* **44(8)**: 2677–268

Glandorf, D. C., Verheggen, P., Jansen, T., Jorritsma, J. W., Smit, E., Lee, F. P., Wernars, K., Thomas, L. S., Laureijis, E., Thomas, J. E., Bakker, P. A. and Van Loon, L. C. 2001. Effect of genetically modified *Pseudomonas putida* WCS358r on the fungal rhizosphere microflora of field-grown wheat. *Applied Environment Microbiology* **67**: 3371-3378

Gross, H. and Loper, J. E. 2009 Genomic of Secondary Metabolite production by *Pseudomonas* spp. *Nature Product Report* **26**:1408-1446

Grossbard, E. 1948. Production of an Antibiotic Substance on Wheat Straw and Other Organic Materials and in the Soil. *Nature London* **161**: 614

Haas, D. and Defago, G. 2005. Biological Control of Soil-borne Pathogens by *Fluorescent pseudomonads. Nature Reviews Microbiology* **3**: 307–319

Hamdali, H., Bouizgarne, B., Hafidi, M., Lebrihi, A., Virolle, M. J. and Ouhdouch, Y. 2008 Screening for Rock Phosphate Solubilizing *Actinomycetes* from Moroccan Phosphate mines. *Applied Soil Ecology* **38**: 12–19

Handelsman, J. and Parke, J. L. 1989. Mechanisms in Biocontrol of Soilborne Plant Pathogens. In Kosuge, T. and Edwin, W. N. (Eds.). *Plant-Microbe Interactions, Molecular and Genetic Perspectives*. New York: McGram-Hill Education

Harman, G. E. and Nelson, E. B. 1994. Mechanisms of Protection of Seed and Seedlings by Biological Control Treatments. In Martin, T. (Eds.). *Implication for Practical Disease Control and Prospects*. British Crop Production Council: Farnham

Harman, G. E. and Kubicek, C. P. 1998. *Trichoderma and Gliocladium*. London: Taylor and Francis

Harman, G. E. 2000. Myths and Dogmas of Biocontrol: Changes in the Perceptions Derived from Research on *Trichoderma harzianum* T-22. *Plant Disease*. **84**: 377-393

He, H., Silo-suh, L. A., Handelsman, J. and Clardy, J. 1994. Zwittermicin A: An Antifungal and Plant Protection Agent from *Bacillus cereus*. *Tetrahedron Letter* **35**: 2499-2502

Henry, M. B., Lynch, J. M. and Fermor, T, R. 1991. Role of Siderophores in the Biocontrol of *Pseudomonas tolaasii* by *Fluorescent pseudomonas* Antagonists. *Journal of Applied Bacteriology* **70**: 104-108

Hiroyuki, T., Hironobu, N., Kazuki, H. and Tsuyoshi, O. 2000. Impact of Siderophore Production on *Pseudomonas aeruginosa* Infections in Immunosuppressed Mice. *Infect Immune* **68(4)**: 1834–1839

Hoch, J., Sonenshein, A. and Losick, A. 1993. *Bacillus subtilis and other Gram-positive Bacteria: Biochemistry, Physiology and Molecular Genetics.* Washington: American Society for Microbiolgy

Ines, M. M. and James, I. P. 2011. Diversity of Endospore-forming Bacteria in Soil Endospore-forming Soil Bacteria. *Soil Biology* **27**: 37-59

Islam, T. M., Hoshidoko, Y., Deora, A., Ito, T. and Tahara, S. 2005. Suppression of Damping-off Disease in Host Plants by the Rhizoplane Bacterium *Lysobacter* sp. strain SB-K88is linked to Plant Colonization and Antibiosis against Soil-borne Peronosporomycetes. *Applied Environment Microbial* **71**:3786-3796

Jayakumar, J., Ramakrishnan, S. and Rajendran, G. 2002. Bio-control of Reniform Nematodes, *Rotylenchulus reniformis* through *fluorencent Pseudomonas. Pesttol* **26**: 45-46

Jayandra, K. J. 2009. *Biological Control of Plant Disease.* New Delhi: New India Publishing Agency

Jiang-Gang, L., Zhi, Q. J., Liu, P. X., Sun, F. F. and Jian-He. G. 2006. Characterization of Chitinase Secreted by Bacillus cereus Strain CH2 and Evaluation of its Efficacy Against *Verticillium* Wilt of Eggplant. *Biology Control* **53**: 931-944

Jinantana, J. and Sariah, M. 1997. Antagonistic effect of Malaysian Isolates of *Trichoderma harzianum* and *Gliocladium Viren* on *Sclerotium rolfsii. Journal of Tropical Agriculture Science* **20**: 35–41

Joanne, M. W., Linda, M. S. and Christopher, J. W. 2011. *Prescott's Microbiology, 8th edition: Isolation of Pure Culture.* New York: McGraw-Hill education

Johri, J. K. 2009. *Recent Advances in Biopesticides.* New Delhi: New India Publishing Agency

Jones, E. E., Mead, A. and Whipps, J. M. 2004. Effect of inoculums type and timing of application of *Coniothyrium minitans* on *Scerotinia sclerotiorum*: control of *Sclerotinia* disease in glasshouse lettuce. *Plant Pathology* **53**: 611-620

Karp, A. and Shield, I. 2008. Bioenergy from Plants and the Sustainable Yield Challenge. *New Phytologist* **179**: 15-32

Katrien, B. D., Bram, S., Willem, W., Paul, V. and Kerr, A. 1980. Biological Control of Crown Gall through Bacteriocin Production. *Journal of Physiological and Molecular Plant Pathology* **4**: 37-44

Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N. 1980. Enhanced Plant Growth Promoting Rhizobacteria. *Nature* **286**: 885-886

Kobayashi, D. Y., Reedy, R. M., Bick, J. A. and Oudemans, P. V. 2002. Characterization of a Chitinase Gene from *Stenotrophomonas maltophilia* strain 34 S1 and its Involvement in Biological Control. *Applied Environment Microbiology* **68**: 1047-1054

Koumoutsi, A., Chen, X. H., Henne, A., Liesegang, H., Gabriele, H., Franke, P., Vater, J. and Borris, R. 2004. Structural and Functional Characterization of Gene Clusters Directing Biocontrol Agents. *Pest Management Science* **59**:475-483

Kurze, S., Bahl, H. and Berg, G. 2001. Biological Control of Fungal Strawberry Disease by *Serratia plymuthica* HRO-C48. *Plant Disease* **85**: 529-534

Kuta, F. A, Mimzing, L. and Orka, A. P. Y. 2009. Screening of *Bacillus* species with potentials of antibiotics production. *Applied Medical Informatics.* **24**: 42-46

Lam, S. T. and Gaffney, T. D. 1993. Biological activities of bacteria used in plant pathogen control. In Chet, I. (Eds.). *Biotechnology in Plant Disease Control*. New York: John Wiley

Laure, W. 2013. The Potential of Bacterial Volatiles for Crop Protection against Phytophathogenic Fungi. In Méndez-Vilas (Eds.). *Microbial Pathogens and Strategies for Combating them*. Switzerland: Science, Technology and Education

Lazarovits, G, and Nowak, J. 1997. Rhizobacteria for Improvement of Plant Growth and Establishment. *Horticulture Science* **32**: 188–192

Lebuhn, M., Heulin, T. and Hartmann, A. 1997. Production of Auxin and other Indolic and Phenolic Compounds by *Paenibacillus polymyxa* Strains Isolated from Different Proximity to Plant Roots. *Federation of European Microbiological Societies Microbiology Ecology* **22**: 325–334

Leclere, V., Bechet, M., Adam, A., Guez, J. S., Wathelet, B., Ongena, M., Thonart, P., Gancel, M. and Jacques,P. 2005. Mycosubtilin overproduction by *Bacillus subtilis* BBG100 Enhance the Organisms Antagonistic and Biocontrol Activities. *Applied Environment Microbial* **71**: 4577-4584

Lemanceau, P. 1992. Beneficial Effects of Rhizobacteria for the Plants, Example: *Pseudomonas fluorescents. Agronomy Journal* **12**: 413–437

Ligon, J. M., Hill, D. S., Hammer, P. E., Torkewitz, N. R., Hofmann, D., Kempf, H. J. and van Pee, K. H. 2000. Natural Product with Antifungal Activity from *Pseudomonas* Biocontrol Bacteria. *Pest Management Science* **56**: 688-695

Liu, L., Kloepper, J. W. and Tuzun, S. 1995. Induction of Systemic Resistance in Cucumber against Bacterial Angular Leaf Spot by Plant Growth-Promoting Rhizobacteria. *Phytopathology* **85**:843-847

Loper, J. E., Kobayashi, D. Y. and Paulsen, I. T. 2007.The Genomic Sequence of *Pseudomonas fluorescens* Pf-5: Insight into Biological Control. *Phytopathology* **97**: 233-238

Lugtenberg, B. and Bloemberg, G. V. 2004. Life in the Rhizosphere.In Ramos, J. L. (Eds.). *Pseudomonas: Genomics, Life Style and Molecular Architecture.* New York: Kluwer

Lynch, J. M. 1990. Introduction: Some Consequences of Microbial Rhizosphere Competence for Plant and Soil. In: Lynch, J. M. (Eds.). *The Rhizosphere*. John Wiley and Sons: Ltd Essex

Maier, T., Klepel, S., Renner, U. and Kostrzewa, M. 2006. Fast and reliable MALDI-TOF MA based Microorganism Identification. *Nature Methods* **3**: 1-2

Manwar, A. V., Khandewal, S. R., Chaudhari, B. L., Meyer, J. M. and Chincholkar, S. B. 2004. Sidespore Production by a Marine *Pseudomonas aeruginosa* and its Antagonistic Action against Phytopathogenic Fungi. *Applied Biochemistry Biotechnology* **118**: 243-251

McInroy, J. A. and Kloepper, J. W. 1995. Survey of Indigenus Bacterial Endophytes from Cotton and Sweet Corn. *Plant and soil* **173**: 337-342

Melanie, P., Ingrid, H., Regina, K. and Ulrich, B. 2013. Application of MALDI-TOF MS for Identification of Food Borne Bacteria. *The Open Microbiology* **7**: 135-141

Mellouli, L., Ameur-Medhi, R. B., Sioud, S., Salem, M. and Bejar, S. 2003. Isolation, Purification and Partial Characterization of Antibacterial Activities Produced by A Newly Isolated *Streptomyces* sp. US24 strain. *Research in Microbiology* **154**: 345– 352

Merriman, P. R., Price, R. D., Kollmorgen, J. F., Piggott, T. and Ridge, E. H. 1974. Effect of Seed Inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the Growth of Cereals and Carrots. *Australian Journal of Agricultural Research* **25**: 219–226

Mervyn, B. and Andrew,H. 2009. *Methods in Enzymology Analyzing the Regulation of Antibiotic Production in* Streptomycetes. Elsevier: Academic Press

Mohammad Reza Safari Motlagh and Zahra Samimi. 2013. Evaluation of *Trichoderma* spp. as Biological Agents in Some of Plant Pathogens. *Annuals of Biological Research* **4(3)**: 173-179

Moyne, A. L., Shelby, R., Cleveland, T. E. and Tuzun, S. 2001. Bacillomycin D: an Iturin with Antifungal Activity against *Aspergillus flavus. Applied Microbiology* **90**: 497-499

Narayanasamy, P. 2013. Biological Management of Disease Crops: Integration of Biological Control Strategies with Crop Disease Management Systems. Berlin: Springer

Pal, K. K. and Gardener, M. G. 2006. Biological Control of Plant Pathogens. *The Plant Health Instructor* **10**: 1117-1120

Palni, P., L. M., Pandey, M. A., Rahman, J., Mahmud Abdul Rahman and Begun, M. M. 2007. Screening of Antagonistic Bacteria for Bio-control Activities on *Colletotrichum gleosporiodes* in Papaya. *Asian Journal Plant S*cience **6**: 12-20

Pandey, R., Singh, H. B. and Kalra, A. 2000. Microbial Management of Plant Parasitic Nematodes. *International Journal of Tropical Plant Disease* **18**:1-23

Parke, J K., Raud, R. E., Joy, A. E. and King, E. B. 1991. Biological Control of *Phythium* damping off and Aphanomyces Root Rot of Peas by Application of *Pseudomonas cepacia* or *P.fluorensces* to Seed. *Plant Disease* **75**: 987-992

Peypoux, F. Bonmatin, J. M. and Wallach, J. 1999. Recent Trends in the Biochemistry of Surfactin. *Applied Microbial Biotechnology* **51**:553-563

Poromarto, S. H., Nelson, B. D. and Freeman, T. P. 1988. Association of Binucleate Rhizoctonia with Soybean and Mechanism of Biological Control of *Rhizoctonia solani. Phytopathology* **88**: 1056-1067

Powell, K. A., and Jutsum, A. R. 1993. Technical and Commercial Aspects of Biological Control Products. *Pesticides Science* **37**: 315-321

Press, C. M., Wilson, M., Tuzun, S., Kloepper, J. W. 1997. Salicylic Acid Produced by *Serratia marcescens* is not the Primary Determinant of Induced Systemic Resistance in Cucumber or Tobacco. *Molecular Plant Microbe Interaction* **10**: 761 -768

Raaijmakers, J. M., Vlami, M. and de Souza, J. T. 2002. Antibiotic Production by Bacterial Biocontrol Agents. *Antonie van Leeuwenhoek* **81**:537-547

Rezzonico, F., Zala, M., Keel, C., Duffy, B., Moënne-Loccoz, Y. and Défago, G. 2007. Is The Ability of Biocontrol *Fluorescent pseudomonads* to Produce the Antifungal Metabolite 2, 4-diacetylphloroglucinol Really Synonymous with Higher Plant Protection? *New Phytolology* **173**: 861–872

Robert, D. P., Kenna, L. F., Lakshman, D. K., Meyer, S. L. F., Kong, H., de Souza, J. T. Lydon, J., Baker, C. J. and Chung, J. S. 2007. Suppression of Damping-off of Cucumber by *Phythium ultimum* with Live Cells and Extracts of *Serratia marcescens* N4-5. *Soil Microbiology and Biochemistry* **39**: 2275-2288

Rosas-Garcia, N. 2009. Biopesticides Production from *Bacillus thuringiensis*: An Environmentally Friendly Alternative. *Recent Patent in Biotechnology* **3**: 28-36

Rumbaugh, K. P., Griswold, J. A., Hamood, A. N. 1999. *Pseudomonas aeruginosa* strains obtained from patients with tracheal, urinary tract and wound infection: variations in virulence factors and virulence genes. *Journal of Hospital Infection* **43**: 211–218

Ryan, R. P., Germaine, K. and Franks, A. 2008. Bacterial Endophytes: Recent Developments and Applications. *Federation of European Microbiological Societies Microbiology Microbiol Letter* **278**: 1-9

Ryu, C. M., Hu, C. H., Reddy M. S. and Kloepper, J. W. 2003. Different Signaling Pathways of Induces Resistance in *Arabidopsis thaliana* Against Two Pathovars of *P.syringe*. *New Phytologist* **16**: 413-420

Ryu, C. M., Kim, J., Choi, O., Kim, SH. and Park, C. S. 2006. Improvement of Biocontrol Capacity *Paenibacillus polymyxa* E681 by Seed Pelleting on Sesame. *Biology Control* **39:** 282-289

Saharan, B.S. and Nehra, V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. *Life Sciences and Medicine Research* **11**: 21

Sandeep, K. M., Pandey, M. and Maheshwarim, D. K. 2009. Reduction in Dose of Chemical Fertilizers and Growth Enhancement of Sesame (*Sesamum indicum* L.) with Application of Rhizospheric Competent *Pseudomonas aeruginosa* LES4. *Euro Journal Soil Biology* **45**: 334-340

Sanford, G. B. 1926. Some Factors Affecting the Pathogenity of Acitinomyces Scabies, *Phytopathology* **16**: 251-547

Santos, V., Barrera, G. C., Miranda, M. A. and Peña, J. J. 2012. *Burkholderia cepacia* XXVISiderophore with Biocontrol Capacity against *Colletotrichum gloeosporioides*. *World Journal of Microbiology and Biotechnology* **28**: 2615-2623

Sarangthem, I. D., Bharat, S., Momota, P. and Narayan C. T. 2012. Genetic Characterization of *Burkholderia cepacia* Strain from Northeast India: A Potential Bio-control Agent. *Advances in Bioscience and Biotechnology* **3**:1179-1188

Saxena, N. P. and Awasthi, D. K. 2003. *Methods for Culturing Microorganisms. Microbiology*. Meerut: Prakashan Media (P) Ltd

Scott, E. M., Rattray, Prisser, A. S., Killham, J. I., Glover,K., Lynch, J. M. and Bazin, M. J. 1995. A Mathematical Model for Dispersal of Bacterial Inoculants Colonizes the Wheat Rhizosphere. *Soil Biology and Biochemistry* **27**:1307-1318

Shanahan, P., Sullivan, D. J., Simpson, P., Glennon, J. D. and O'Gara, F.1992. Isolation of 2,4-diacetylphloroglucinol from a *Fluorescent pseudomonas* and Investigation of Physiological Parameters Influencing its Production. *Applied Environment Microbial* **58**: 353-358

Shaukat, S. S., Siddiqui, I. A., Hamid, M., Khan, G. H. and Asli, S. A. 2002. *In vitro* Survival and Nematicidal Activity of *Rhizobium, Bradyrhizobium* and *Sinorhizobium*. The Influence of Various NaCl Concentrations. *Journal of Biology Science* **5**: 669-671

Shishido, M., Loeb, B. M., Chanway, C. P. 1995. External and Internal Root Colonization of Lodgepole Pine Seedlings by Two Growth-Promoting *Bacillus* Strains Originated from Different Root Microsites. *Canadian Journal of Microbiology* **41**: 707–713

Shouan, Z., Thomas. L. W., Miriam, C. M., John, A., Joseph, W. K. and Waldemar, K. 2010. Evaluation of Plant Growth-Promoting Rhizobacteria for Control of Phytophthora blight on Squash under Greenhouse Conditions. *Biological Control* **53**: 129-135

Singh, A., Parmar, N., Ramesh, C. K. and Owen, P, W. (eds.). 2011. Bioaugmentation, Biostimulation, Biocontrol. Berlin: Springer

Someya, N. N., Kataoka, T., Komagata, T., Hibi, K. and Akutsu. 2000. Biological Control of Cyclamen Soil Borne Diseases by *Serratia marcescens* strain B2. *Plant Disease* **84**: 334-340

Spaepen, S., Vanderleyden, J. and Remans, R. 2007. Indole-3-Acetic Acid in Microbial and Microorganism-Plant Signaling. *Federation of European Microbiological Societies Microbiology Review **31**:425–448*

Sticher, L., Mauch, M. and Metraux, J. P. 1997. Systemic Acquired Resistance. *Annual Review Phytopathology* **35**: 235-270

Surette, M. A., Sturza, V., Lada, R. R. and Nowak, J. 2003. Bacterial Endophytes in Processing Carrots (*Daucus carota L.var.*sativus): Their Localization, Population Density, Biodiversity and Their Effects on Plant Growth. *Plant Soil* **253**: 381-390

Susanto, A., Sudharto, P. S. and Purba, R.Y. 2005. Enhancing Biological Control of Basal Stem Rot Disease (*Ganoderma boninese*) in Oil Palm Plantation. *Mycopathologia*, **159**: 153-17

Sylvia, K. G., Milton, N. S., John, J. C., Spyros, D. K. and Vilma, B. V. 1975. Agricultural Plants and Soil as a Reservoir for *Pseudomonas aeruginosa. American Society for Microbiology* **29(6)**: 987-991

Tahvonen, R. and Lahdenpera, M. L. 1988. Biological Control of *Botrytis cinerea* and *Rhizoctonia solani* in Lettuce by *Steptomyces* sp. *Annual Agriculture Fennicae* **27**: 107-116

Timmusk, S., Nicander, B., Granhall, U. and Tillberg, E. 1999. Cytokinin Production by *Paenibacillus polymyxa. Soil Biology and Biochemistry* **31**: 1847–1852

Tom, C. and Peter, V. 2003. Diversity and Significance of *Burkholderia* Species occupying Diverse Ecological Niches. *Environmental Microbiology* **5(9)**: 719–729

Trivedi, B., Trivedi, P., Pandey, A. and Palni, L. M. 2008. In *vitro* Evaluation of Antagonistic Properties of *Pseudomonas corrugate. Microbiological Research* **163**: 329-336

Tschen, J. S. and Tseng, C. N. 1989. An Antifungal Antibiotic Isolated from Metabolites of *Bacillus* cereus CHU 130. *Life Sciences* **13 (4)**: 258–61

Vessey J. K. 2003. Plant Growth Promoting Rhizobacteria as Biofertilizers. *Plant Soil* **255**: 571-586

Wakayama, S., Ishikawa, F. and Oishi, K. 1984. Mycocerein, A Novel Antifungal Peptide Antibiotic Produced by *Bacillus cereus. Antimicrobial Agents Chemotherapy* **26**: 939–940

Weid, I., Artursson, V., Seldin, L. and Jansson, J. K. 2005. Antifungal and Root Surface Colonization Properties of GFP-tagged *Paenibacillus brasiliensis* PB177. *World Journal Microbiology Biotechnology* **12**: 1591- 1597

Weindling, R. 1934. Studies on Lethal Principle Effective in the Parasitic Action of *Trichoderma lignorum* on *Rhizoctonia solani* and Other Soil Fungi. *Phtyopathology* **24**: 1153-1179

Weller, D. M. 1988. Biological Control of Soilborne Plant Pathogens in the Rhizosphere with Bacteria. *Annual Review Phytopathology* **26**: 379-407

Weller, D. M. and Thomashow, L. S. 1993. Microbial metabolites with Biological Activity against Plant Pathogens. In Lumsden, R. D. and Vaughn, J. L. (Eds.). *Pest Management: Biologically Based Technologies.* Washington: American Chemical Society

Whipps, J. M., 1990. Carbon utilization .In Lynch, J. M. (Eds.). *The Rhizosphere.* Wiley Interscience: United Kingdom

Whipps, J. M. 2001. Microbial Interaction and Biocontrol in the Rhizosphere. *Journal of Experimental Botany* **52**: 487-511.

Wiese, A., Munstermann, M., Gutsmann, T., Lindner, B., Kawahara, K., Zahringer, U. and Seydel, U. 1998. Molecular Mechanisms of Polymyxins B-membrane Interactions: Direct Correction between Surface Change Density and Self-promoted Transport. *Journal of Membrane Biology* **82**: 127-138

Wilhite, S. E., Lunsden, R. D. and Strancy, D.C. 2001. Peptide synthetase gene in *Trichoderma* virens. *Applied Environmental Microbiology* **67**: 5055-5062

Williams, T. L., Andrzejewski, D., Lay, J.O. and Musser, S. M. 2003. Experimental Factors Affecting the Quality and Reproducibility of MALDI TOF Mass Spectra obtained from Whole Bacteria Cells. *Journal of the American Society for Mass Spectrometry* **14 (4)**: 342–351

Woo, S. L., Donzelli, B., Scala, F., Harman, G. E., Kubicek, C. P., Sorbo, G. and Lorito, M. 1999. Disruption of the ech42 (endochitinaseencoding) Gene Affect Biocontol Activity in *Trichorderma harzianum* P1. *Molecular Plant-Microbe Interaction* **12**: 419-429

Yang, J., Kharbanda, P. D. and Mirza, M. 2004. Evaluation of Paenibacillus Polymyxa pkb 1 for Biocontrol of Phythium Disease of Cucumber in a Hydroponic System. *Acta Horticulturae* **635**: 59-66

Zaiton, S., Sariah, M. and Zainal, A. M. A. 2008. Effect of Endophytic Bacteria on Growth and Suppression of Ganoderma Infection in Oil Palm. *International Journal of Agricultural and Biology* **10**: 127-132

Zehder, G. W., Yao, C., Murphy, J. F., Sikora, E. J. and Kloepper, J. W. 2000. Induction of Resistance in Tomato against Cucumber Mosaic Cucumovirus by Plant Growth-Promoting Rhizobacteria. *Biocontrol* **45**: 127–137