Chapter 12
Bio (Bacterial) Control of Pre- and Postharvest Diseases of Root and Tuber Crops

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12.1 Introduction

There are approximately 400 vegetable crops (including root and tuber crops) that are commercially cultivated worldwide (Kays and Silva Dias 1996). The term "root and tuber crops" is a very general "catch-all" for a wide cross section of subterranean storage organs of which there are approximately 38 root, 23 tuber, 14 rhizome, 11 corm, and 10 bulb crops. Crops with an enlarged pseudostem or stem (e.g., leek, kohlrabi), even when subterranean, are generally not considered within the root and tuber crop category. Likewise, each of the crops included are commercially cultivated and marketed, though in some instances the volume is not great; species that are gathered from the wild are not included. All of the crops are utilized as food though in diverse ways: e.g., as staples, vegetables, sources of industrial products and condiments. Root and tuber crops are divided, for convenience, into temperate (i.e., potato (*Solanum tuberosum* L.), sugar beet (*Beta vulgaris* L.), onion (*Allium cepa* L.) and garlic (*Allium sativum* L.), etc.) and tropical (i.e., cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas* L.), yams (*Dioscorea* spp.), and edible aroids (*Colocasia esculenta* L.) Schott. and *Xanthosoma* spp.), based on the climate in which they are cultivated. Global productions of some of the important root and tuber crops are given in Table 12.1 (FAO 2003).

Pre- and postharvest losses of these crops are very high and, depending on the species cultivated and the storage environment, may be of the order of 30–60% during the course of 3–6 months (Proctor et al. 1981). The principle causes of loss include (1) weight loss due to desiccation, (2) loss of carbohydrate and water due to

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Table 12.1 Production statistics for selected root and tuber crops in 2002

Crop	Production (Metric tones/year)
Beet (sugar)	246,475,609
Carrot	21,020,436
Cassava	184,852,540
Chicory roots	960,700
Garlic	12,107,007
Ginger	988,182
Onion (dry)	51,914,247
Potato	307,440,446
Sweet potato	136,130,396
Taro	9,220,522
Yam	39,643,170

Source: FAO (2003)

respiration, (3) sprouting on breakage of dormancy, (4) losses due to rodents and insects, and (5) fungal, bacterial, and viral diseases. Biological control involving microorganisms is currently used to prevent various diseases, particularly fungal and to some extent bacterial diseases on seed potatoes, sugar beet, yams, and aroids (Lebot 2008).

This chapter focuses on the progress made in the recent years on the spectrum of bacteria used as antagonists for control of pre- and postharvest diseases of these crops, their modes of action, and methods to enhance biocontrol efficacy of the antagonists.

12.2 Microbial Control Strategies

There are two basic approaches for using microbial antagonists for controlling plant diseases:

1. Manipulation of epiphytic and endophytic microflora
2. Those that can be artificially introduced against pathogens.

12.2.1 Manipulation of Epiphytic and Endophytic Microflora

Identification and selection of effective antagonistic bacteria is the first and foremost step in biological control. Antagonistic bacteria are mostly searched among endophytic and epiphytic microflora of cultivated plants. However, the implication of endophytic bacteria in biological control has received less attention as compared to epiphyte bacteria (Helbig 2006). Few representative examples are cited below to reinforce this view.

Rhizoctonia solani causes stem, stolon canker and black scurf on potato tubers, reducing plant health, yield quality and quantity (Rauf et al. 2007). Lahlali et al. (2007) reported that endophyte and non-endophyte bacterial isolates from healthy potato plants and rhizosphere for their antagonistic activity against *R. solani*. Among a total of 220 bacterial isolates, only 25 showed a highly significant inhibition rate against *R. solani* in in vitro dual culture assays.

Pseudomonas putida, originally isolated from the tuber surface (geocaulosphere) of potato showed in vitro antibacterial activity to the bacterial pathogen, *Erwinia carotovora*. Similarly, the T4 lysozyme sensitive *Serratia grimesii* isolated from the rhizosphere of parental potatoes showed in vitro antagonism toward *Verticillium dahliae*. Both introduced isolates were able to colonize the rhizo- and geocaulosphere of transgenic plants and non-transgenic parental plants and established in the rhizosphere at 10^5 colony forming units/g fresh weight of root (Lottmann et al. 2000). These strains also significantly decreased Fusarium dry rot of potato at cell concentrations of 10^6 cells/ml (Lottmann et al. 1999). Various antagonistic bacteria were isolated from the phylloplane of onion crops. Among epiphytic microorganisms, *Bacillus amyloliquefaciens* BL-3, *Paenibacillus polymyxa* BL-4, and *Pseudomonas putida* Cha 94 were highly inhibitory to conidial germination of fungi such as *Fusarium oxysporum*, *Aspergillus* sp., and *Botrytis allii*, which were the basal and neck rot causing pathogens for onion during storage (Lee et al. 2001). Likewise, application of *Bacillus subtilis*, isolated from the epiphytic microflora of yam tuber, showed a drastic reduction in the number of spoilage fungi of yams during 5-month storage period. The surface fungi like *Botryodiplodia theobromae*, *Fusarium moniliforme*, and *Penicillium sclerotigenum* were displaced completely on the treated tubers (Okigbo 2002, 2005).

12.2.2 Introduction of Microbial Antagonists

Earliest efforts to control plant diseases involved the introduction of antagonistic bacteria, *Bacillus subtilis* and *Pseudomonas cepacia* and fungi, *Trichoderma* and *Rhodotorula* (Sadfi et al. 2001, 2002; Sharma et al. 2009) in plant–pathogen interactive environment. Since then, several other antagonists (including yeasts) have been identified and used for controlling various plant diseases. Table 12.2 shows exclusively the bacterial control of pre- and postharvest diseases of root and tuber crops.

12.2.2.1 Fungi

Fungi are also used as antagonist in disease control of roots and tubers. Studies showed that *Trichoderma reesei* and *T. viride* significantly reduced the incidence of *Rhizoctonia* symptoms on potato sprouts. *Gliocladium* (*Trichoderma*) *virens*,

Table 12.2 Microbial antagonists used for control of diseases of roots and tuber crops

Antagonists	Disease and pathogen	Crop(s)	Reference(s)
Bacillus licheniformis	Botrytis rot (*Botrytis allii*)	Onion	Lee et al. (2001)
Bacillus amyloliquefaciens	Fusarium rot (*Fusarium oxysporum*)		
Bacillus spp.	Fusarium rot (*Fusarium roseum* var. *sambucinum*)	Potato	Sadfi et al. (2002)
Bacillus subtilis	Sugar beet Cercospora leaf spot (*Cercospora beticola*)	Sugar beet	Collins and Jacobsen (2003)
Bacillus subtilis	Botryodiplodia rot (*Botryodiplodia theobromae*)	Yams	Okigbo (2002), Swain et al. (2008)
Bacillus subtilis	Fusarium rot (*Fusarium moniliforme*)	Yams	Okigbo (2002)
Pantoea agglomerans	Dry rot (*Gibberella pulicans*)	Potato	Schisler et al. (2000)
Pseudomonas fluorescens	Dry rot (*Gibberella pulicans*)	Potato	Schisler et al. (2000)
Pseudomonas sp.	Blue and Green rot (*Penicillium sclerotigenum*)	Yams	Okigbo (2002)
Pseudomonas fluorescens	Soft rot (*Erwinia carotovora* subsp. *atroseptica*)	Potato	Cronin et al. (1997)
Bacillus cereus, *Cellulomonas fimi*, *Kocuria varians*, *Pseudomonas putida*, *Rhodococcus erythropolis*, *Rhodococcus globerulus*	Silver scurf (*Helminthosporium solani*)	Potato	Martinez et al. (2002)
Burkholderia cepacia	Dry rot (*Fusarium sambucinum*, *Fusarium oxysporum* and *Fusarium culmorum*)	Potato	Al-Mughrabi (2010)
Pseudomonas putida	*Erwinia carotovora* ssp. *atroseptica*	Potato	Lottmann et al. (2000)
Pseudomonas fluorescens	*Pythium ultimum*	Sugar beet	Thrane et al. (2000)

Fusarium oxysporum, *F. lateritium*, *Penicillium tritinum*, and *Taralomyces* spp. have good potential for potato cyst nematode biocontrol (Nagtzaam and Bollen 1997; Sharma et al. 2009; Indarti et al. 2010).

From in vitro and in vivo screening tests for antagonism by isolates of *Trichoderma* against postharvest pathogens of yams (*Dioscorea* spp.), an isolate of *Trichoderma viride* was selected as the most promising candidate. Inoculation of

white yam (*Dioscorea rotundata* Poir.) with conidia of *T. viride* and subsequent storage of the tubers under the ambient environmental conditions of a traditional yam barn resulted in drastic reduction in the frequency of occurrence of the normal tuber surface mycoflora (*Aspergillus niger*, *Botryodiplodia theobromae* and *Penicillium oxalicum*) over a 4-month storage period (Okigbo and Ikediugwu 2008).

12.2.2.2 Yeast

Yeasts are mostly used for control of postharvest diseases of fruits, vegetables, roots, and tubers. Tian et al. (2005) have made several positive points in recommending yeasts as potential microbial agents for controlling the postharvest diseases including (a) they can colonize the wound surface for long period even under dry conditions; (b) they produce extracellular polysaccharides, which enhance their survivability and restrict the growth of pathogen propagules; (c) they can use nutrients rapidly and proliferate at a faster rate; and (d) they are the least affected by the pesticides. Of the various yeasts, *Candida sake*, *Candida albidus*, *Candida oleophila*, *Debaryomyces hansenii*, *Pichia anomala*, *Pichia guilliermondii*, *Issatchenkia orientalis*, *Metschnikowia pulcherrima*, *Cryptococcus laurentii*, etc., have exhibited a wide spectrum of biological activity against many postharvest plant pathogens (Sharma et al. 2009).

12.2.2.3 Bacteria

Bacterial flora have attracted enormous attention as agents for biocontrol plant diseases, particularly since they are easy to handle, generally stable, resistant, and ability to survive desiccation and inherently possess a quick generation time (Sharma et al. 2009). They are also known to affect life cycles of different plant pathogens or pests by diverse mechanisms including the production of extracellular metabolites and intracellular proteinaceous toxins. In general, spore-forming bacteria (e.g., *Bacillus* spp.) survive to a greater extent even in harsh environments, compared to the non-spore-forming bacteria. Among the *Bacillus* spp., the ones that have attracted the most attention are *Bacillus thuringiensis*, *B. amyloliquefaciens*, *B. licheniformis*, and *B. subtilis* (Swain et al. 2008; Al-Mughrabi 2010). Other bacterial species of interest are *Pseudomonas fluorescens* (Thrane et al. 2000), *Pseudomonas putida* (Sharma et al. 2009), *Talaromyces flavus* (Nagtzaam and Bollen 1997), *Pantoea agglomerans* (Kim et al. 2006), etc. Bacteria are easily mass produced using a liquid fermentation process, although in some cases they may be more amenable to semi-solid or solid-state fermentation (Swain and Ray 2008).

12.3 The Mode of Action of Bacterial Agents

Biological control by bacteria uses naturally occurring mechanism to suppress plant pathogens. The modes of action are antibiosis, competition for space, nutrients, siderophore-mediated suppression, parasitism, cell-wall lytic enzymes, and induced systemic resistance (Sharma et al. 2009). In general, more than one mechanism is implicated, but in no case was a single mechanism found to be responsible/suitable for biological control.

12.3.1 Antibiosis

Production of antibiotics is the most important mechanism by which the bacterial antagonists suppress the pathogens (Fig. 12.1). Examples of antibiotics are iturins (a powerful antifungal peptide) produced by *Bacillus subtilis*, pyrrolnitril produced by *Pseudomonas cepacia*, trichothecenes produced by *Myrothecium roridium*, etc., (Bull et al. 1998).

Pseudomonas fluorescens DR54, isolated from sugar beet rhizosphere, had shown biocontrol of *Pythium* in planta (Nielsen et al. 1998). This bacterial isolate produced the antifungal cell-associated lipopolypeptide, viscosinamide (Nielsen et al. 1999), which induced physiological changes in *Pythium ultimum* and *Rhizoctonia solani* in vitro and in soil as studied by fluorescent microscopy (Hansen et al. 2000). Viscosinamide was detected in increasing amounts on both seed coats and in rhizosphere soil surrounding the sugar beet roots during 7 days of incubation (Thrane et al. 2000). In another study, *Pseudomonas aeruginosa* PNA1 strongly reduced root rot disease tissue culture derived cocoyam plantlets. Soil experiments involving the strain PNA1 in comparison to phenazine-deficient mutants suggested that the biocontrol activity of PNA1 against *Pythium myriotylum* might involve phenazines. Phenazine involvement was further strengthened by the fact that FM13 fed with exogenous tryptophan (so that phenazine production was restored) reduced disease severity on cocoyam. The efficiency of PNA1 to control *P. myriotylum* on cocoyam was improved when the strain and the pathogen were allowed to interact for 24 h prior to transplanting cocoyam plantlets, while doubling the inoculum density of the pathogen negatively affected its efficiency (Tambong and Hofte 2001).

Recep et al. (2009) showed that *Burkholderia cepacia* OSU-7 has great potential to be used as biocontrol agents for management of *Fusarium* species causing dry rot on potato. *Burkholderia cepacia*, formerly known as *Pseudomonas cepacia* (Yabuuchi et al. 1992), produces one or more antibiotics that are active against a broad range of plant pathogenic fungi (Rosales et al. 1995). Organisms of the *B. cepacia* complex produce inhibitory metabolites such as pyrrolnitrin (Hwang et al. 2002), siderophores (Stephan et al. 1993), cepaciamide A(B), cepacidine A(B), cepacin A(B), and lipopeptides (Kang et al. 1998). These antibiotics appear,

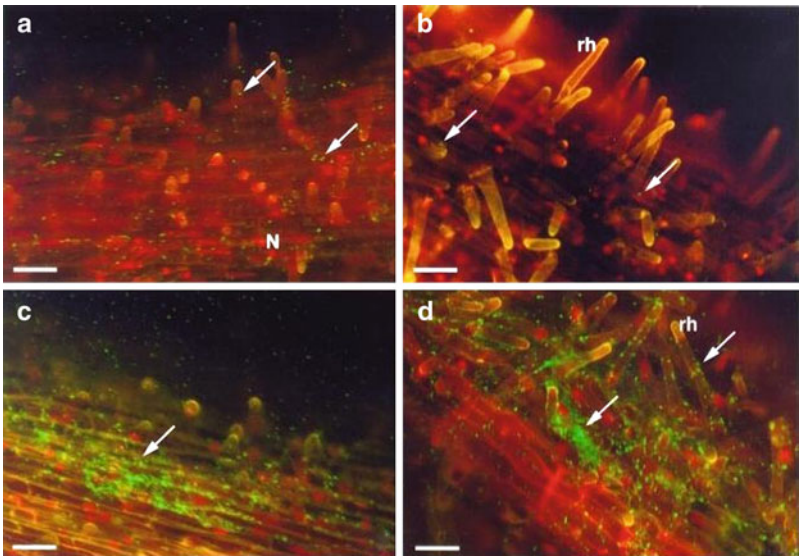

Fig. 12.1 Confocal microscopy image of carrot roots in the region of (**a**) and (**c**), emerging and, (**b**) and (**d**), fully developed root hairs. *Pseudomonas fluorescens* are visible as *small green spots* on the root surface. Plant nuclei are stained in *red*. Differences in the abundance of bacterial cells were observed when the wild-type CHA0 (**a** and **b**) or the mucoid mutant CHA211 (**c** and **d**) were used in the assay. *N* nucleus, *rh* root hair. *Bars* = 30 μm (Hansen et al. 2000)

in many cases, to be important for disease suppression. Compounds such as cepacin A and cepacin B exhibit only antibacterial activity, whereas pyrrolnitrin is effective against fungi, yeasts, and Gram-positive bacteria (Quan et al. 2006).

12.3.2 Siderophore Production

Effect of root-colonizing *Pseudomonas* to enhance crop yields is partly due to siderophore produced by them that make iron in the rhizosphere less available to deleterious fungi and rhizobacteria (Nautiyal et al. 2006; Pandey et al. 2005). Siderophores are low-molecular weight compounds synthesized under iron-deficient conditions by many microorganisms. They chelate Fe^{3+} and the resulting iron complex is transported into the cell through receptor mediation. The siderophores of fluorescent pseudomonads are commonly referred to as pseudobactins or pyroveridines.

12.3.3 Competition for Nutrients

Competition for nutrients is the most promising mode of action for several bacterial agents. Attachment by bacterial antagonist to the pathogen hyphae appears to be an important factor necessary for competition for nutrients as shown by the interactions

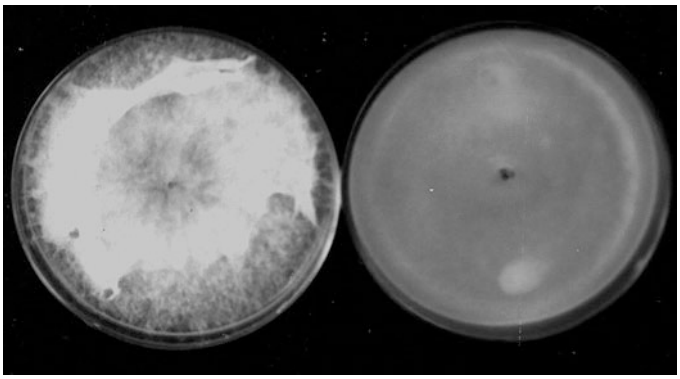

Fig. 12.2 Competition for Space between yam postharvest pathogen *Botryodiplodia theobromae* and biocontrol agent *Bacillus subtilis*. (**a**) control (*B. theobromae*), (**b**) treatment (*B. theobromae* + *B. subtilis*) (Swain and Ray 2009a)

of *Pseudomonas fluorescens* and *Pythium utimum* in sugar beet (Thrane et al. 2000). In vitro studies conducted on such interactions revealed that due to direct attachment, antagonistic bacteria take nutrients more rapidly than target pathogens and thereby prevent spore germination and growth of the pathogens. Nonpathogenic species of *Erwinia*, such as, *E. cypripedii*, showed antagonistic activity against various isolates of *Erwinia caratovora* sub sp. *caratovora*, the causal agent of soft rot of carrot, by competing for nutrients (Sharma et al. 2009).

12.3.4 Competition for Space

Competition for space is the competition for infection sites, which may occur if antagonists are able to occupy the specific places where recognition mechanisms between host and pathogen take place (Fig. 12.2). If these places are no more available for pathogens, the necessary procedure of recognition cannot take place and infection does not occur. Thus, microbial antagonists should have the ability to grow more rapidly than the pathogen (Lübeck et al. 2000).

Lübeck et al. (2000) studied *P. fluorescens* DR54 colonization of the sugar beet rhizosphere by confocal laser scanning microscopy and found that *P. fluorescens* DR54 was the dominating organism a few days after the inoculation. During their 20 days study, active micro-colonies of *P. fluorescens* DR54 could be detected on all parts of the roots. Lottmann et al. (2000) demonstrated that isolates of *P. putida* QC14-3-8 and L16-3-3 were able to colonize potato tuber surface of transgenic as well as that of non-transgenic potatoes, but the culturable population of both inoculants was about one exponential unit lower in the geocaulosphere than in the rhizosphere. This was expected, because the rhizosphere represents a more attractive habitat compared to the tuber surface due to the exudation of nutrients (Lottmann et al. 1999)

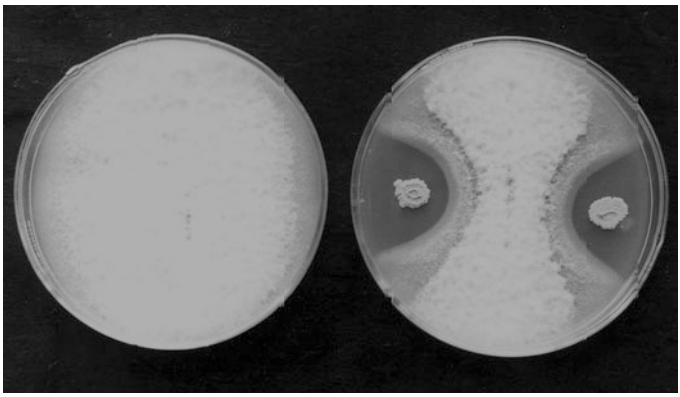

Fig. 12.3 Antibiotic activity between yam postharvest pathogen *Fusarium oxysporum* and biocontrol agent *Bacillus subtilis*. (**a**) control (*F. oxysporum*), (**b**) treatment (*B. theobromae* + *B. subtilis*) (Swain and Ray 2009a)

12.3.5 Production of Cell-wall Lytic Enzymes

Microbial antagonists also produce lytic enzymes such as gluconase, chitinase, and proteinases that help in the cell wall degradation of the pathogenic fungi (Chernin and Chet 2002). The interaction between *Bacillus subtilis* and *Fusarium oxysporum*, the postharvest pathogens of yam (*Dioscorea* spp.) tubers, was studied by scanning electron microscopy (Swain et al. 2008). Lysis of fungus cell wall by *B. subtilis* was observed owing to the production of extracellular chitinase (Fig. 12.3).

12.3.6 Direct Parasitism

Antagonist and pathogen can interact also through a direct parasitism. Bianciotto et al. (2001) tested the biofilm forming ability of two mutant strains with increased production of acidic extracellular polysaccharides compared with the wild-type biocontrol strain *Pseudomonas fluorescens* CHA0. The anchoring of bacteria to axenic non-mycorrhizal and mycorrhizal roots of carrot as well as on extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* was investigated. The non-mucoid wild-type strain *P. fluorescens* CHA0 adhered very little on all surfaces, whereas both mucoid strains formed a dense and patchy bacterial layer on the carrot roots and fungal structures. Increased adhesive properties of plant-growth-promoting bacteria may lead to more stable interactions in mixed inocula and the rhizosphere.

12.3.7 Induce Resistance

Interactions between plants and pathogens can lead either to a successful infection (compatible response) or resistance (incompatible response). In incompatible interactions, infection by viruses, bacteria, or fungi will elicit a set of localized responses in and around the infected host cells. These responses include an oxidative burst (Lamb and Dixon 1997), which can lead to cell death (Kombrink and Schmelzer 2001). Thus, the pathogen may be "trapped" in dead cells and appears to be prevented from spreading from the site of initial infection. Further local responses in the surrounding cells include changes in cell wall composition that can inhibit penetration by the pathogen and de novo synthesis of antimicrobial compounds such as phytoalexins and pathogenesis related (PR) proteins (Hammerschmidt 1999).

Bacillus mycoides isolate Bac J (BmJ) was capable of inducing systemic acquired resistance (SAR) in sugar beet (Bargabus et al. 2002). Two molecular markers associated with pathogen-induced SAR, β-1,3-glucanase, and chitinase were found to be induced during BmJ–plant interactions. These host responses could, therefore, be used as a predictor of systemic resistance induction capability, provided the assay results are both accurate and precise (Bargabus et al. 2004).

12.4 Fields of Use for Bacterial Antagonists

Bacteria are generally more predominant microflora in nature, compared to yeasts and filamentous fungi. A large body of information is available concerning antagonism between bacteria and pathogenic fungi.

12.4.1 Seed Treatment

When seeds are treated with antagonists, the antagonist is present from the beginning of plant growth. Due to early presence antagonists are able to colonize the seed and the young roots successfully. Competition by other inhabitants of the rhizosphere is significantly reduced and antagonists are able to establish a high population density. *Bacillus subtilis* and *Pseudomonas* spp. are the antagonists mainly investigated for this purpose (Schmiedeknecht et al. 1998). For example, seed treatment of potatoes with *B. subtilis* successfully reduced incidence of *Rhizoctonia solani* and *Streptomyces scabies* in greenhouse and field trials. Reduction of disease incidence in field trials varied between different experimental years but reached a level of up to 50% for *R. solani* and up to 67% for *S. scabies* (Schmiedeknecht et al. 1998). Similarly, *Pseudomonas fluorescens–putida* and strain R20 of *P. putida*, when inoculated onto sugar beet seed, resulted in a markedly lower incidence of colonization by *Pythium ultimum*. The incidence of fungal colonization of beet seeds treated with *P. fluorescens* or *P. putida* was 6.7 and 35.7%, respectively, compared with 90% of untreated seeds after planting (Osburn et al. 1982).

12.4.2 Soil Application

Application of antagonists to soil has many advantages. The most important one might be the relatively low variation that occurs in soil environmental conditions. Extreme events such as heavy rainfall and dryness due to several hours of sunshine are buffered by soil. Therefore, antagonists have less stress and higher possibility of survival after application (Helbig 2006).

Extracellular polysaccharides play an important role in the formation of bacterial biofilms. Bianciotto et al. (2001) tested the biofilm forming ability of two mutant strains with increased production of acidic extracellular polysaccharides compared with the wild-type biocontrol strain of *P. fluorescens*. The anchoring of bacteria to axenic nonmycorrhizal and mycorrhizal carrot roots as well as on extra radical mycelium of the arbuscular mycorrhizal fungus, *Glomus intraradices*, was investigated. The non-mucoid wild-type strain *P. fluorescens* adhered very little on all surfaces, whereas both mucoid strains formed a dense and patchy bacterial layer on the carrot roots and fungal structures (Fig. 12.4).

12.4.3 Treatment of Aerial Plant Parts

Biological control of plant pathogens on aerial plant parts obviously is the most challenging task for microbial antagonists. Regarding crops in the field, aerial plant parts are exposed to environmental conditions such as heavy rainfalls, dryness, UV-radiation, wind, and other conditions. These unfavorable conditions hinder survival of large antagonist populations for longer periods. Foliar application of *Psudomonas fluoresens* and *P. putida* strain significantly reduced the number of angular leaf spots per leaf on susceptible clones in cassava (Hernandez et al. 1985).

12.5 Measures to Improve Performance of Antagonists

There are several approaches to improve the performance of antagonists; some representative examples are described below.

12.5.1 Addition of Nutrients

The addition of nutrients is aimed at increasing the performance of antagonists by providing nutrients. Entry et al. (2000) hypothesized that wood chip-polyacrylamide cores surrounding host plant roots could alter the soil environment to favor growth of introduced biocontrol microorganisms (*Streptomyces lydicus* or *Pseudomonas corrugata*), thereby reducing *Verticillium dahliae* infection of potato in greenhouse condition.

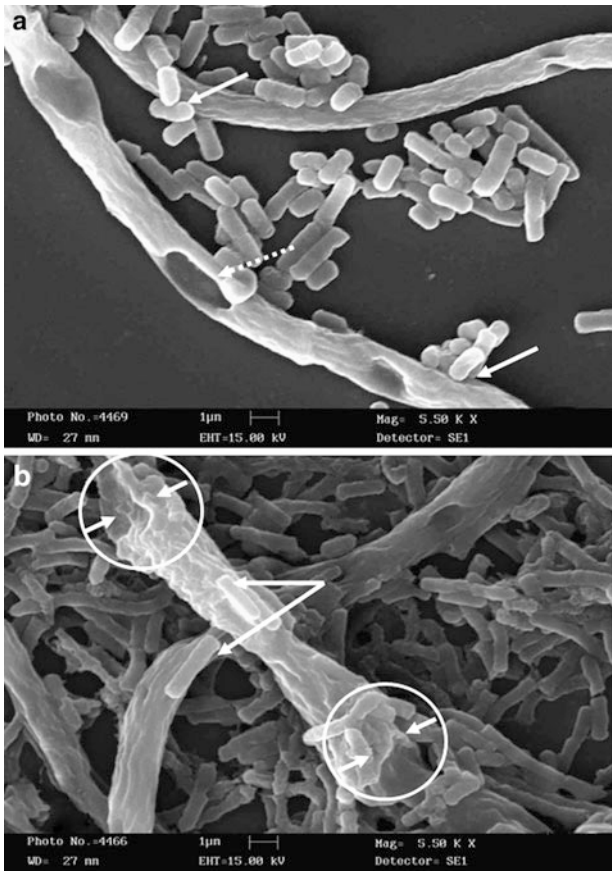

Fig. 12.4 Scanning electron micrograph of *Fusarium oxysporum* sample collected at 12 h (**a**) and 36 h (**b**) after interaction with *Bacillus subtilis* CM1. The *solid* and *dotted arrow* shows the bacterial attachment with fungal hyphae and lytic mark hyphae. *Circles* indicate the complete lysis of fungal mycelium after 36 h of interaction (Swain et al. 2008)

12.5.2 Use of Antagonist Mixtures

Because of the different sensitivities of fungi, yeasts and bacteria vis-à-vis environmental conditions, especially on aerial plant parts, the use of antagonist mixtures could contribute to consistency of performance of antagonist preparations. If one organism fails due to unfavorable conditions, the others should be effective under these conditions. Moreover, the combination might have a synergistic effect.

Pseudomonas fluorescens F113 and *Stenotrophomonas maltophilia* W81 when combined in a consortium improved the level of protection in comparison when used singly to protect sugar beet from *Pythium*-mediated damping-off (Dunnea et al. 1998). In another study, commercial biocontrol agents, microbial inoculants,

mycorrhizae, and an aerobic compost tea (ACT), were used in three different 2-year crop rotations (barley/ryegrass, barley/clover, and potato, all followed by potato). Biological amendments reduced soil-borne disease and improved yield in some rotations, but not others. Soil-applied ACT and the combination of ACT with a mixture of beneficial microorganisms (Mix) reduced stem canker, black scurf, and common scab on potato tubers by 18–33% and increased yield by 20–23% in the barley/ryegrass rotation, but not in the other rotations. Mix also reduced disease (20–32%) in the barley/clover rotation only (Larkin 2008).

12.5.3 Formulation of Antagonists

Formulation of antagonists should fulfill a variety of functions with the overarching goal to support viability and by this stabilize and increase performance of antagonists.

Seed treatment with non-sterilized powdered straws from 39 crops was tested for the control of *Pythium* damping-off of sugar beet. Four straws, including flax, coriander, pea, and lentil were effective in controlling the disease in soil artificially infested with *Pythium* sp. Sterilizing flax and pea straws eliminated the efficacy of these straws. Wheat straw powder coated on sugar beet seeds increased the incidence of *Pythium* damping-off, but this effect was reversed by the co-inoculation of wheat straws with the biocontrol agent *Pseudomonas fluorescens* 708. Coating sugar beet seeds with *P. fluorescens* 708 and flax or pea straws also increased the efficiency of the bacterial strain for the control of *Pythium* damping-off. Pea straws and to a lesser extent lentil straws produced volatile substances that affected mycelial growth of *Pythium* sp. (Bardin et al. 2004).

12.6 Bacterial Biocontrol for Root and Tuber Crops

Bacteria are the most promising biocontrol agents as compared to the fungi and yeast. Most of the fungal infections in root and tuber crops are controlled by bacteria-based biocontrol agents.

12.6.1 Temperate Root Crops

Plants cultivated for its swollen edible roots (which may or may not be a tuber roots) are called root and tuber crops. Crops that are mostly cultivated in temperate climate such as carrot, potato, beet root, etc., are called temperate tubers crops.

12.6.1.1 Potato

Potato tubers endophytic bacteria were examined from the tuber peel (periderm plus top 3 mm of tissue) of four cultivars (Russet Burbank, Kennebec, Butte, and Shepody). Endophytic bacteria from several layers of peel were challenged in in vitro bioassays to the soil-borne plant pathogens, *Fusarium sambucinum*, *Fusarium avenaceum*, *Fusarium oxysporum*, and *Phytophthora infestans* (mating types A1 and A2). In general, antibiosis of bacterial endophytes against these pathogens was significantly higher in isolates recovered from the outermost layer of tuber peel and decreased progressively toward the center of the tuber. Antibiosis against *P. infestans* was variable, with a progressive decrease in antibiotic activity from outer to inner layers of peel occurring in cultivars, "Russet Burbank" and "Kennebec," only. In all cases the inhibitory activity of endophytic bacteria was significantly greater against the A1 than the A2 mating type of *P. infestans*. In four of seven cases, where the same species of bacteria were recovered from all three peel layers, antibiosis to pathogens decreased significantly with depth of recovery (from the periderm to inside the tuber), indicating that in certain communities of endophytic bacteria, defense against pathogens may be related to bacterial adaptation to location within a host and may be tissue-type and tissue-site specific (Sturz et al. 1999).

Eight plant growth promoting rhizobacteria (PGPR) of different species (*Bacillus subtilis*, *Bacillus pumilus*, *Burkholderia cepacia*, *Pseudomonas putida*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus macerans*, and *Flavobacter balastinium*) were tested for antifungal activity in in vitro (on Petri plate) and in vivo (on potato tuber) conditions against *Fusarium sambucinum*, *F. oxysporum*, and *F. culmorum* that cause dry rot of potato. All PGPR strains were also tested on tubers of two potato cultivars "Agria" and "Granola" under storage conditions. Only, *B. cepacia* strain OSU-7 had significant effects on controlling potato dry rot caused by three different fungi (Al-Mughrabi 2010).

Bacillus thuringiensis, previously selected for their efficiency against insects, were tested in vitro and in vivo against *Fusarium roseum* var. *sambucinum*, the causal agent of dry rot of potato tubers. Results of the in vitro dual culture screening revealed that more than 50% of *Bacillus* spp. isolated from salty soils inhibited the growth of the pathogen in vitro. By contrast, other *B. thuringiensis* strains failed to inhibit the growth of the pathogen in vitro. On wounded potato tubers, the most effective isolates obtained from salty soils were X7, X9, X16, I32, and G7 Bacillus strains, with a percentage of dry rot reduction ranging from 66 to 89%. These effective *Bacillus* isolates were identified as belonging to one of the species *Bacillus cereus* (X9, X16 and G7), *B. lentimorbus* (X7), or *B. licheniformis* (I32). Although ineffective in vitro, *B. thuringiensis* strains inhibited dry rot development in vivo, with percentage inhibition scores ranging from 41 to 52%. While *Bacillus* isolates selected from salty soils best inhibited dry rot development when applied as young cultures (24 h), *B. thuringiensis* strains generally performed better as older cultures (48–72 h). The cell filtrates of *Bacillus* spp. were unable to inhibit the growth of *Fusarium*. By contrast, volatiles liberated by the antagonists seem to contribute to the inhibition of the pathogen (Sadfi et al. 2001).

The antagonistic potential of potato- (endophytic and ectophytic) associated, a total of 2,648 bacteria were screened by dual testing of antagonism to the soil-borne pathogens, *Verticillium dahliae* and *Rhizoctonia solani*. The rhizosphere and endorhiza were the main reservoirs for antagonistic bacteria and showed the highest similarity in their colonization by antagonists. The most prominent species of all microenvironments was *Pseudomonas putida*, and rep-PCR with BOX primers showed that these isolates showed microenvironment-specific DNA fingerprints. *P. putida* isolates from the rhizosphere and endorhiza gave nearly identical fingerprints confirming the high similarity of bacterial populations. The *phlD* gene, involved in the production of the antibiotic 2,4-diacetyl-phloroglucinol, was found only among *Pseudomonas* isolates from the rhizosphere and endorhiza. Evaluation of the bacterial isolates for biocontrol potential based on fungal antagonism and physiological characteristics resulted in the selection of five promising isolates from each microenvironment. The most effective isolate was *Serratia plymuthica* 3Re4-18 isolated from the endorhiza (Berg et al. 2005).

Twenty-eight potential biocontrol organisms were tested for efficacy against *Rhizoctonia solani* on potato in a series of greenhouse trials. Organisms tested consisted of field isolates of *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Penicillium* sp., *Trichoderma* sp., and *Rhizoctonia zeae*; known biocontrol isolates including *Laetisaria arvalis*, *Verticillium biguttatum*, *Cladorrhinum foecundissimum*, and *Stilbella aciculosa*; and commercial products of *Bacillus subtilis* (Kodiak), *Trichoderma virens* (SoilGard), and *T. harzianum* (RootShield). Different formulations and rates of several fungal isolates and the efficacy of combinations of effective antagonists were also investigated. None of the treatments, including a chemical control (azoxystrobin), effectively controlled stem canker and black scurf in all trials. However, *B. subtilis* GB03, *R. zeae* LRNE17E, *S. aciculosa* 112-B, and the chemical control were most effective in reducing stem canker severity (40–49% reduction) relative to the infested controls over all trials. *L. arvalis* ZH-1, *R. zeae* LRNE17E, and the chemical control reduced black scurf severity 54–60% relative to the infested control. Other treatments also significantly reduced stem canker and black scurf; however, they were slightly less effective. Biocontrol amendment rate was not correlated with disease control, although the higher rates usually provided the best control. One combination of biocontrol organisms, *B. subtilis* and *T. virens*, demonstrated somewhat better control of stem canker than each organism alone, suggesting that this approach may provide improved biocontrol efficacy (Brewer and Larkin 2005).

12.6.1.2 Sugar Beet

Bacillus subtilis isolate, BacB, is extensively studied for the control of sugar beet Cercospora leaf spot, caused by *Cercospora beticola* Sacc. by examining application timing, biocontrol agent (BCA) concentration, use of the selective nutrient substrate β-glucan, and the form of the BCA at the time of application. Examining the effects of varying β-glucan concentrations and levels of BacB at application

demonstrated a complex interaction between β-glucan, BCA population, and disease control. Growth chamber experiments demonstrated that applying the bacteria as vegetative cells instead of spores or applying the BCA 1–5 days before infection could significantly increase disease control. Laboratory experiments demonstrated the ability to induce germination and vegetative growth of BacB from a spore formulation, without shaking or fermentation equipment. This shows promise for optimizing *Bacillus* sp. for biological control. In field trials the vegetative cells did not perform better than the spore application, though the potential for β-glucan to increase disease was demonstrated (Collins and Jacobsen 2003).

Pseudomonas fluorescens F113 and *Stenotrophomonas maltophilia* W81 protect sugar beet from *Pythium*-mediated damping-off through production of the antifungal secondary metabolite 2,4-diacetylphloroglucinol and extracellular proteolytic activity, respectively. Growth and in vitro production of 2,4-diacetylphloroglucinol by F113 and extracellular lytic enzymes by W81 were not affected when inoculated in combination. The abilities of W81 and F113 to colonize the rhizosphere of sugar beet were essentially similar when the two strains were applied singly or co-inoculated onto seeds in a 1:1 ratio, both in natural soil microcosms and under field conditions. Concomitantly, single inoculation with W81 or F113 effectively prevented colonization of sugar beet seeds by *Pythium* spp. in soil microcosms, without the necessity for combining both strains. However, this parity was not reflected in seed emergence where the combination of W81 and F113 significantly enhanced final sugar beet stands (to the level achieved with chemical pesticides) under microcosm conditions at 28 days after sowing. In a field experiment, the only inoculation treatment capable of conferring effective protection of sugar beet was that in which W81 and F113 were co-inoculated, and this treatment proved equivalent to the use of chemical fungicides. In conclusion, when compared with single inoculations of either biocontrol strain, the combined use of a phloroglucinol-producing *P. fluorescens* and a proteolytic *S. maltophilia* improved protection of sugar beet against *Pythium*-mediated damping-off (Dunnea et al. 1998).

In another study, dual compatibility of antagonists (*Pseudomonas* and *Bacillus* strains) were also evaluated against *Pythium* spp. Antagonist combinations did not show any superior biocontrol ability to individual antagonists and compatibility of bacteria in vitro did not correlate with compatibility in vivo (Georgakopoulos et al. 2002).

Bacillus mycoides isolate Bac J., a nonpathogenic, phyllosphere-inhabiting bacterium, reduces Cercospora leaf spot (*Cercospora beticola* Sacc.) of sugar beet by 38–91% in both glasshouse and field experiments. Disease control was attributed to the bacterium's ability to induce systemic resistance, which was demonstrated through classical induced resistance challenge experiments, western analysis, and enzyme activity assays. Enzyme assays following *B. mycoides* and acibenzolar-*S*-methyl treatment demonstrated increased activity of chitinase, β-1,3-glucanase, and peroxidase, all pathogenesis-related proteins and accepted indicators of systemic resistance. Western analysis detected numerous chitinase isoforms in *B. mycoides* and acibenzolar-*S*-methyl-treated plants that were not detected in the water controls. The active chitinase isoforms were identified using in-gel activity assays.

β-1,3-glucanase activity assays following native polyacrylamide gel electrophoresis revealed two unique isoforms produced following *B. mycoides* treatment, one of which was also found with acibenzolar-*S*-methyl treatment. The increase in peroxidase-specific activity following acibenzolar-*S*-methyl and *B. mycoides* treatment was due to production of two unique isoforms not found in the water-treated plants as shown by activity assays following native polyacrylamide gel electrophoresis (Bargabus et al. 2002).

Pseudomonas fluorescens–putida and strain R20 of *P. putida*, when inoculated onto seed, resulted in a markedly lower incidence of colonization by *P. ultimum*. The incidence of fungal colonization of beet seeds treated *P. fluorescens–putida* or *P. putida* was 6.7 and 36.7%, respectively, compared with 90% of untreated seeds 24 h after planting. *P. fluorescens* inhibited both mycelial growth and sporangial germination, whereas *P. putida* inhibited only mycelial growth (Osburn et al. 1982).

Pseudomonas fluorescens (biovars I to VI) were collected from the rhizosphere of field-grown sugar beet plants to select candidate strains for biological control of preemergence damping-off disease. The isolates were tested for in vitro antagonism toward the plant-pathogenic microfungi *Pythium ultimum* and *Rhizoctonia solani* in three different plate test media. Mechanisms of fungal inhibition were elucidated by tracing secondary-metabolite production and cell wall-degrading enzyme activity in the same media. Most biovars expressed a specific mechanism of antagonism, as represented by a unique antibiotic or enzyme production in the media. A lipopeptide antibiotic, viscosinamide, was produced independently of medium composition by *P. fluorescens* bv. I, whereas the antibiotic 2,4-diacetylphloroglucinol was observed only in glucose-rich medium and only in *P. fluorescens* bv. II/IV. Both pathogens were inhibited by the two antibiotics. Finally, in low-glucose medium, a cell wall-degrading endochitinase activity in *P. fluorescens* bv. I, III, and VI was the apparent mechanism of antagonism toward *R. solani*. The viscosinamide-producing DR54 isolate (bv. I) was shown to be an effective candidate for biological control, as tested in a pot experiment with sugar beet seedlings infested with *Pythium ultimum*. The assignment of different patterns of fungal antagonism to the biovars of *P. fluorescens* was discussed in relation to an improved selection protocol for candidate strains to be used in biological control (Nielsen et al. 1998).

The effectiveness of *Bacillus subtilis* isolate, in the field and growth chamber in the presence of the fungus, *Cercospora beticola*, was studied. The use of the selective biocontrol agent support substrate β-glucan, applied at 0, 0.5, and 1.0% of the spray solution, did not influence differences in total population numbers (spores + vegetative cells) of a spontaneous rifampicin resistant isolate of BacB (Rif+) over a 14-day spray period. BacB Rif+, applied as a spore formulation, declined from 10,000 CFU (Colony Forming Units)/cm^2 on day 0.5–100 CFU/cm^2 on day 14 at the three levels of β-glucan tested. Distribution of BacB Rif + populations was modeled on a leaf scale, with and without β-glucan. Higher populations of vegetative cells were more likely at 14 days with 1% β-glucan than with 0% β-glucan. BacB populations were more aggregated without β-glucan than with the nutrient substrate. There was no correlation between BacB density and Cercospora leaf spot disease severity, indicating that neither antibiosis nor parasitism is likely an important mechanism of disease control (Collins and Jacobsen 2003).

Nontarget effects of a bacterial (*Pseudomonas fluorescens* DR54) and a fungal (*Clonostachys rosea* IK726) microbial control agent (MCA), on the indigenous microbiota in bulk soil and rhizosphere of barley, and subsequently a sugar beet crop, were studied in a greenhouse experiment. MCAs were introduced by seed and soil inoculation. Bulk and rhizosphere soils were sampled regularly during the growth of barley and sugar beet. The soils were assayed for the fate of MCAs and various features of the indigenous soil microbiota. At the end of the experiment (193 day), DR54 and IK726 had declined by a factor of 106 and 20, respectively, and DR54 showed a short-lasting growth increase in the sugar beet rhizosphere. In general, the nontarget effects were small and transient. IK726 seemed to have general stimulating effects on soil enzyme activity and the soil microbiota, and resulted in a significant increase in plant dry weight. The plant growth promoting effect of DR54 was less pronounced and the DR54 displaced indigenous pseudomonads. DR54 stimulated growth of protozoans with a tolerance for the antifungal compound viscosinamide produced by DR54. Treatment with the fungicide Fungazil had no effects on plant growth or soil microorganisms. Phospholipid fatty acid (PLFA) analysis detected the perturbations of the soil microbial community structure in the MCA treatments as well as the return to non-perturbed conditions reflecting the decline of inoculant populations. The PLFA technique appeared to be suitable for in situ monitoring of MCA nontarget effects on the soil microbiota, but should be combined with assays for MCA survival and soil enzyme activity (Johansen et al. 2005).

Growth inhibition of the root pathogen *Pythium ultimum* by the biocontrol agent *Pseudomonas fluorescens* DR54 inoculated on sugar beet seeds was studied in a soil microcosm. Plant emergence was followed, together with bacterial rhizosphere colonization, antibiotic production, and effects on fungal growth. *P. fluorescens* DR54 inoculation of the *P. ultimum*-challenged seeds improved plant emergence after 7 days compared to a control without the biocontrol strain. At this time, *P. fluorescens* DR54 was the dominating colony-forming pseudomonad in rhizosphere soil samples from inoculated seedlings as shown by immuno-staining with a strain-specific antibody. Viscosinamide, which has previously been identified as a major antagonistic determinant produced by *P. fluorescens* DR54 and shown to induce physiological changes in *P. ultimum* in vitro, could be detected in the rhizosphere samples. The impact of *P. fluorescens* DR54 on the growth and activity of *P. ultimum* was studied by direct microscopy after staining with the vital fluorescent dyes Calcofluor white and fluorescein diacetate. *P. fluorescens* DR54 caused reduction in *P. ultimum* mycelial density, oospore formation, and intracellular activity. Further, *Pythium* oospore formation was absent in the presence of *P. fluorescens* DR54. A striking effect on zoospore-forming indigenous fungi was also observed in microcosms with *P. fluorescens* DR54 and, thus, where viscosinamide could be detected; a large number of encysted zoospores were seen in such microcosms both with and without *P. ultimum* infections. In vitro studies confirmed that purified viscosinamide-induced encystment of *Pythium* zoospores (Thrane et al. 2000).

12.6.1.3 Carrot

Diseased carrot seeds were treated with selected microorganisms isolated from soils, carrot seeds, and tap roots. The effects of those antagonists on the control of *Alternaria radicina* were evaluated by growing-on tests on water agar, filter paper, vermiculite, and in a potting medium. The germination percentage, emergence percentage, and the disease severity of those carrot seeds treated with *Burkholderia* (*Pseudomonas*) *cepacia* no.229 were significantly ($P = 0.05$) differed from the nontreated seeds and the seed treated with other antagonists. The effects of *B. cepacia* no.229 in promoting seed emergence and controlling disease were as good as those seeds treated with iprodione (100 ppm). Black rot lesions on carrot tap roots were significantly reduced ($P = 0.05$) in size when roots were treated with *B. cepacia* no 229 or *Bacillus amyloliquefaciens* no. 224 compared to the nontreated roots. Also, *B. cepacia* no. 229 significantly ($P = 0.05$) reduced black rot on the foliage of carrot compared to check (Chen and Wu 1999).

12.6.1.4 Onion

Bacillus subtilis (B-2) in the rhizospheres of onion seedlings grown from bacterized seeds in muck soil at various pH, moisture, and temperature regimes were monitored for 14 weeks. Seed bacterization significantly increased shoot dry weight (12–94%), root dry weight (13–100%), and shoot height (12–40%) of onion seedlings over controls. Increases in shoot height and shoot weight were greatest at low temperature and high moisture, under all pH regimes. Root weight was similarly affected by temperature and moisture, but was significantly increased at pH 6.5 compared to 5.5 and 4.5. Though *B. subtilis* B-2 failed to maintain high populations in the onion rhizosphere, it nevertheless caused significant growth effects on bacterized onion seedlings. The observed growth effects were not proportional to rhizosphere populations of B-2 (Reddy and Rahe 1989).

Bacillus amyloliquefaciens BL-3 and *Paenibacillus polymyxa* BL-4 were applied in the rhizoplane of onion at transplanting. BL-3 completely suppressed the neck rot of onion (Lee et al. 2001). Further, strain BL-3 produced a heat-stable antifungal protein (Bae 1999) that reduced decay regardless of the inoculum level or the isolate of pathogenic fungi tested and was effective at temperatures of 10–30 °C (Lee et al. 2001).

12.6.1.5 Ginger

Kahili ginger (*Hedychium gardnerianum*) is an invasive weed in tropical forests in Hawaii and elsewhere. Bacterial wilt caused by the ginger strain of *Ralstonia* (=*Pseudomonas*) *solanacearum* systemically infects edible ginger (*Zingiber officinale*) and ornamental gingers (*Hedychium* spp.), causing wilt in infected plants.

The suitability of *R. solanacearum* as a biological control agent for kahili ginger was investigated by inoculating seedlings and rooted cuttings of native forest plants, ornamental ginger, and solanaceous species to confirm host specificity. Inoculation via stem injection or root wounding with a bacterial–water suspension was followed by observation for 8 weeks. Inoculations on *Hedychium gardnerianum* were then carried out in ohia-lehua (*Metrosideros polymorpha*) wet forests of Hawaii Volcanoes National Park to determine the bacterium's efficacy in the field. No native forest or solanaceous species developed wilt or other symptoms during the study. The bacterium caused limited infection near the inoculation site on *H. coronarium*, *Z. zerumbet*, *Heliconia latispatha*, and *Musa sapientum*. However, infection did not become systemic in any of these species, and normal growth resumed following appearance of initial symptoms. All inoculated *H. gardnerianum* plants developed irreversible chlorosis and severe wilting 3–4 weeks following inoculation. Systemic infection also caused death and decay of rhizomes. Most plants were completely dead 16–20 weeks following inoculation. The destructiveness of the ginger strain of *R. solanacearum* to edible ginger has raised questions regarding its use for biological control. However, because locations of kahili ginger infestations were often remote, the risk of contaminating edible ginger plantings was unlikely. The ability of this bacterium to cause severe disease in *H. gardnerianum* in the field, together with its lack of virulence in other ginger species, contributed to its potential as a biological control agent (Anderson and Gardner 1999).

12.6.1.6 Garlic

Treatment of garlic cloves with tebuconazole achieved a significant reduction in the rate of disease progress and the final incidence of plant death by *Sclerotium cepivorum*; garlic yields were improved. In contrast, lower levels of disease control were obtained when selected isolates of *Trichoderma harzianum* and *Bacillus subtilis* were applied to the soil and cloves, respectively (Melero-Vara et al. 2000).

Pantoea agglomerans S59-4 isolate from rhizosphere or rhizoplane of *Allium* species was selected as a potential biocontrol agent against *Penicillium hirsutum*, when using an in vivo wounded garlic bulb assay. When the spore suspension (10^5 spores/ml) of *P. hirsutum* was co-inoculated with a cell suspension of *P. agglomerans* S59-4 (10^8 CFU/ml) isolate on wounded garlic, the isolate showed a highly suppressive effect on disease development. Bacterial density of *P. agglomerans* on wounded garlic cloves increased continuously both under room temperature and low temperature conditions until 30 days after application. In addition, *P. agglomerans* showed in vitro inhibitory effects against various postharvest diseases of citrus fruits, apples, onions, lettuces, and carrots. In particular Pa59-4 showed strong inhibitory effects against *Penicillium digitatum*, *Aspergillus niger*, *Sclerotinia sclerotiorum*, and *Geotrichum candidum* (Kim et al. 2006).

12.6.2 Tropical Tuber Crops

Tropical roots and tuber crops are important staples for food security for about a fifth of the world crop production. The most important tropical root and tuber crops include cassava, yam, cocoyam, sweet potato, colocasia (taro), and amorphophallus.

12.6.2.1 Cassava

Forty isolates of fluorescent *Pseudomonas* were isolated from the plants growing in five different ecosystems in Nigeria. Thirty-four of these isolates inhibited *Erpinia aarotovoroa* pv. *aarootovoroa*, in vitro, the causal agent of cassava stem rot. One-month old plantlets, produced by rooting the shoots of a cultivar in distilled water, were inoculated with suspensions (1×10^9 cells/ml) of each *Pseudomonas* isolate. Inoculated plants were free from symptoms of root pathogens and roots swelled earlier than controls. Microbial deterioration of bulked swollen roots was also reduced up to 60% when roots were dip treated in a bacterial suspension (1×10^9 cells/ml) of the above isolates and stored for 15 days in polyethylene bags. Taxonomic studies showed that these bacterial isolates were either *Pseudomonas putida* (90%) or *P. fluorescens* (10%) (Hernandez et al. 1985).

Cassava bacterial blight caused by *Xanthomonas campestris* pv *manihot's* is a serious problem in the cassava growing region of Nigeria. Several bacterial anatagonists such as *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas* spp., and some fungi such as *Trichoderma* spp. and *Gliocadium* spp. were applied successfully to control the blight (Amusa and Odunbaku 2007).

Sixty-seven endophytic bacteria isolated from cassava cultivated by Brazilian Amazon Indian tribes were subjected to 16S rRNA sequencing and FAME (fatty acid methyl ester) analysis. The bacterial profile revealed that 25% of all endophytic isolates belonged to the genus *Bacillus*. The isolate *B. pumilus* MAIIIM4a showed a strong inhibitory activity against the fungi *Rhizoctonia solani*, *Pythium aphanidermatum*, and *Sclerotium rolfsii* causing cassava stem rot. Secondary metabolites of this strain were extracted using hexane, dichloromethane, and ethyl acetate (Pereira de Melo et al. 2009).

12.6.2.2 Yam

The leaf spot of yam (*Dioscorea cayennensis* Lam.), caused by *Curvularia eragrostidis* (Henn.) Meyer, is one of the most frequent and severe diseases in all yam growing areas of northeast Brazil. The disease causes a reduction of about 35–40% of the weight of the commercial tuber (Michereff et al. 1994). From a total of 162 bacterial isolates, 39 showed antagonism to the pathogen. The bacteria produced extracellular, nonvolatile, and diffusible metabolites in the membrane cellophane test. Seventeen isolates resulted in more than 75% inhibition of

C. eragrostidis mycelial growth. Among them, IF-26 showed the greatest antagonism. The isolates IF-82, IF-88, and IF-109 inhibited pathogen conidial germination, with average inhibition levels of 99.2, 98.2, and 96.2%, respectively. Under greenhouse conditions the antagonists were applied at three different time intervals relative to *C. eragrostidis* inoculation: 3 days before, at the same time, and 3 days after. IF-82 and IF-88 applied at the same time as pathogen inoculation both reduced disease severity to the extent of 75%. IF-82 showed the best persistence of antagonistic action, with an average of 96.3%. IF-82, identified as *Bacillus subtilis*, was the best biocontrol agent for the yam leaf spot disease in this study (Michereff et al. 1994).

Rot of yam tubers and setts may be caused by a wide variety of fungi including *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium solani*, *Penicillium* spp. *Rhizopus stolonifer*, and *Mucor* spp. Twenty-four yam rhizobacteria were screened on potato dextrose agar–nutrient agar (PDA–NA) plates for antifungal activity toward the above rot fungi using the zone of inhibition test. The most promising bacterial antagonist was tested further against 22 fungi from different phyla. Nine rhizobacterial isolates, representing 38% of all bacterial isolates initially tested, exhibited antifungal activity. They were all Gram-negative rods, catalase positive, aerobic, endospore-forming rods, and tentatively identified as *Bacillus* spp. (Awuah and Akrasi 2007).

Bacillus subtilis (Enrenberg) Cohn was investigated for its antagonistic properties against surface mycoflora of yam (*Dioscorea rotundata* Poir) tubers in storage. Yam tubers inoculated with a spore suspension of *B. subtilis* in potato dextrose broth using a knapsack sprayer showed a drastic reduction in the range and number of mycoflora, including pathogens of the tuber surface in contrast to the control tubers, during the 5-month storage period in a traditional yam barn. However, *B. subtilis* maintained a high frequency of occurrence during the same period. *Botryodiploidia theobromae* Pat, *Fusarium moniliforme* Wollen and Reink., *Penicillium sclerotigenum* Yamamoto, and *Rhizoctonia* sp. were displaced completely on the treated tubers. The antagonism of *B. subtilis* was so effective that the normal tuber surface mycoflora was greatly reduced throughout the storage period of 5 months by a simple initial application of the antagonist (Okigbo 2002).

The biocontrol potential of *Bacillus subtilis* isolated from cow dung microflora was investigated in vitro and in vivo against two postharvest yam pathogenic fungi, *Fusarium oxysporum* and *Botryodiplodia theobromae*. *B. subtilis* strains inhibited the growth of *F. oxysporum* and *B. theobromae* in vitro in liquid medium in the range of 49.3–56.6% and in solid medium in the range of 31.0–36.0%, in comparison to the corresponding growth of fungi without bacterial inoculation. The interaction between *B. subtilis* CM1 and *F. oxysporum* was also studied by scanning electron microscopy (Fig. 12.3). In vivo study showed that *B. subtilis* strains inhibited the growth of fungi (*F. oxysporum* and *B. thobromae*) up to 83% in wound cavities of yam tubers (Swain et al. 2008). Likewise, these strains blocked the production of oxalic acid produced by these pathogenic fungi in yam tuber as well as in culture medium (Swain and Ray 2009b).

12.6.2.3 Cocoyams

Root rot of cocoyam (*Xanthosoma sagittifolium*) caused by *Pythium myriotylum* is the most devastating disease of this important tropical tuber crop with yield reductions of up to 90%. *Pseudomonas aeruginosa* PNA1 (wild type) produced phenazine-1-carboxylic acid and phenazine-1-carboxamide (oxychlororaphin), while its tryptophan auxotrophic mutant FM13 was phenazine negative and secreted anthranilate in vitro (Tambong and Hofte 2001). PNA1 and FM13 significantly inhibited growth of *P. myriotylum* in dual cultures, while their supernatants highly reduced mycelial dry weight in potato dextrose broth. However, in the presence of tissue culture derived cocoyam plantlets, only strain PNA1 strongly reduced root rot disease severity. Soil experiments involving strain *P. aeruginosa* PNA1 in comparison to phenazine-deficient mutants suggested that the biocontrol activity of PNA1 against *P. myriotylum* might involve phenazines. Phenazine involvement was further strengthened by the fact that FM13 fed with exogenous tryptophan (so that phenazine production was restored) significantly reduced disease severity on cocoyam. The efficiency of PNA1 to control *P. myriotylum* on cocoyam was significantly improved when the strain and the pathogen were allowed to interact for 24 h prior to transplanting cocoyam plantlets, while doubling the inoculum density of the pathogen negatively affected its efficiency (Tambong 2000). *Pseudomonas* CMR5c and CMR12a were identified as novel and promising biocontrol agents of *P. myriotylum* on cocoyam, producing an arsenal of antagonistic metabolites (Perneel et al. 2007). *Pseudomonas aeruginosa* PNA1 was considered as a promising biological control agent to solve the increasing problem of cocoyam root rot in Cameroon (Tambong and Hofte 2001).

12.6.2.4 Sweet Potato

Endophytic bacteria associated with sweet potato plants (*Ipomoea batatas* L. Lam.) were isolated, identified, and tested for their ability to fix nitrogen, produce indole acetic acid (IAA), and exhibit stress tolerance. Eleven such strains belonging to the genera, *Enterobacter*, *Rahnella*, *Rhodanobacter*, *Pseudomonas*, *Stenotrophomonas*, *Xanthomonas*, and *Phyllobacterium*, were identified (Khan and Doty 2009). Other bacterial species have been reported from sweet potato endophytic included *Acetobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, and *Pseudomonas* (Lodewyckz et al. 2002). These bacteria were associated with plant growth promoting activity and biological control of pathogens in sweet potato (Katarina et al. 2005).

12.7 Conclusion and Future Perspectives

Despite our understanding of the mechanisms by which bacterial antagonists offer disease resistance to root and tuber crops, the ecological significance of their presence as endophytes or epiphytes and in rhizosphere and phyllosphere is less understood. These factors need to be addressed sufficiently in order to develop biocontrol products of commercial interest. Further, most of the studies on biocontrol of diseases are concentrated on potato and sugar beet, and very fewer attentions have been given to other tuber crops particularly on tropical root crops like cassava and sweet potato. When considering possible improvements in biological plant protection, formulation of antagonists, use of such formulations in seed coating and aerial spray, and use of antagonists mixture and in combination with known and established biocontrol enhancing additives such as $CaCl_2$, NaCl are to be studied.

References

Al-Mughrabi KI (2010) Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. Biol Control 53:280–284

Amusa NA, Odunbaku OA (2007) Biological control of bacterial diseases of plants in Nigeria: problems and prospects. Res J Agric Biol Sci 3:979–982

Anderson RC, Gardner DE (1999) An evaluation of the wilt-causing bacterium *Ralstonia solanacearum* as a potential biological control agent for the alien kahili ginger (*Hedychium gardnerianum*) in Hawaiian forests. Biol Control 15:89–96

Awuah RT, Akrasi KO (2007) Suppression of tuber rot of yam caused by *Aspergillus niger* with a yam rhizosphere. Afr Crop Sci Conf Proc 8:875–879

Bae DW (1999) Purification and characterization of an antifungal protein from *Paenibacillus macerans* PM-1 antagonistic to rice blast fungus, *Pyricularia oryzae* Cavara. Ph.D. Thesis. Gyeongsang National University, Chinju, 85 p

Bardin SD, Huang HC, Moyer JR (2004) Control of *Pythium* damping-off of sugar beet by seed treatment with crop straw powders and a biocontrol agent. Biol Control 29:453–460

Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ (2002) Characterization of systemic resistance in sugar beet elicited by a non-pathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. Physiol Mol Plant Pathol 61:289–298

Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ (2004) Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. Biol Control 30:342–350

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol 51:215–229

Bianciotto V, Andreotti S, Balestrini R, Bonfante P, Perotto S (2001) Mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. Mol Plant Microbe Interact 14:255–260

Brewer MT, Larkin RP (2005) Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. Crop Prot 24:939–950

Bull CT, Wadsworth MLK, Sorenson KN, Takemoto J, Austin R, Smilanick JL (1998) Syringomycin E produced by biological agents controls green mold on lemons. Biol Control 12:89–95

Chen TW, Wu WS (1999) Biological control of carrot black rot. J Phytopathol 147:99–104
Chernin L, Chet I (2002) Microbial enzymes in the biocontrol of plant pathogens and pests. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, New York
Collins DP, Jacobsen BJ (2003) Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet cercospora leaf spot. Biol Control 26:153–161
Cronin D, Moënne-Loccoz Y, Fenton A, Dunne C, Dowling DN, Gara FO (1997) Ecological interaction of a biocontrol *Pseudomonas fluorescens* strain producing 2,4-diacetylphloroglucinol with the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica*. FEMS Microbiol Ecol 23:95–106
Dunnea C, Moenne-Loccoza Y, McCarthya J, Higginsa P, Powellb J, Dowlinga DN, O'Gara F (1998) Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of Pythium-mediated damping-off of sugar beet. Plant Pathol 47:299–307
Entry JA, Strasbaugh CA, Sojka RE (2000) Wood chip-polyacrylamide medium for biocontrol bacteria decreases *Verticillium dahliae* Infection on potato. Biocontrol Sci Technol 10:677–686
FAO (2003) FAOSTAT Data Base. Food and Agriculture Organization of the United Nations, Rome, http://apps.fao.org/page/collect.ions?subset=agriculture
Georgakopoulos DG, Fiddaman P, Leifert C, Malathrakis NE (2002) Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimum* with bacterial and fungal antagonists. J Appl Microbiol 92:1078–1086
Hammerschmidt R (1999) Induced disease resistance: how do induced plants stop pathogens. Physiol Mol Plant Pathol 55:77–84
Hansen M, Thrane C, Sorensen J, Olsson S (2000) Confocal imaging of living fungal hyphae challenged with the antifungal antagonist. Mycologia 92:216–221
Helbig J (2006) Microbial control of plant pathogens of horticultural crops–an overview. In: Ray RC, Ward OP (eds) Microbial biotechnology in horticulture, vol 1. Science, New Hampshire, NE, pp 345–396
Hernandez JM, Laberry R, Lozano, JC (1985) Biocontrol of cassava (*Manihot esculenta*) root rots by fluorescent *Pseudomonas*. In: VIIth Sympsoium of international society for tropical root crops, Gosier (Guadeloupe)
Hwang J, Chilton WS, Benson DM (2002) Pyrrolnitrin production by *Burkholderia epacia* and biocontrol of Rhizoctonia stem rot of poinsettia. Biol Control 25:56–63
Indarti S, Widianto D, Kim YH, Mulyadi S (2010) Survey of egg- and cyst-parasitic fungi of potato cyst nematode in Indonesia. Plant Pathol J 26:32–36
Johansen A, Knudsen IMB, Binnerup SJ, Winding A, Johansen JE, Jensen LE, Andersen KS, Svenning MM, Bonde TA (2005) Non-target effects of the microbial control agents *Pseudomonas fluorescens* DR54 and *Clonostachys rosea* IK726 in soils cropped with barley followed by sugar beet: a greenhouse assessment. Soil Biol Biochem 37:2225–2239
Kang Y, Carlson R, Tharpe W, Schell MA (1998) Characterization of genes involved in biosynthesis of a novel antibiotic from *Burkholderia cepacia* BC11 and their role in biological control of *Rhizoctonia solani*. Appl Environ Microbiol 64:3939–3947
Katarina C, Hojka K, Ravnikar M (2005) Bacterial endophytes from seeds of Norway spruce (*Picea abies* L. Karst). FEMS Microbiol Lett 244:341–345
Kays SJ, Silva Dias JC (1996) Cultivated vegetables of the world. Exon, Athens
Khan Z, Doty SL (2009) Characterization of bacterial endophytes of sweet potato plants. Plant Soil. doi:10.1007/s11104-009-9908-1
Kim HJ, Kim YK, Kim TS, Park KS, Yeh WH (2006) Biological control of postharvest diseases by *Pantoea agglomerans* 59-4 on garlic bulbs. In: 27th International horticulture congress exhibition (IHC 2006), 13–19 Aug 2006, COEX (Convention & Exhibition), Seoul, Korea, p 494
Kombrink E, Schmelzer E (2001) The hypersensitive response and its role in local and systemic disease resistance. Eur J Plant Pathol 107:69–78
Lahlali R, Bajii M, Jijakli MH (2007) Isolation and evaluation of bacteria and fungal as biocontrol agents against *Rhizoctonia solani*. Commun Appl Biol Sci Ghent Univ 72:973–982
Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol 48:251–275

Larkin PR (2008) Relative effects of biological amendments and crop rotations on soil microbial communities and soil borne diseases of potato. Soil Biol Biochem 40:1341–1351

Lebot V (2008) Tropical root and tuber crops cassava, sweet potato, yams and aroids. CAB International, Cambridge, MA

Lee JT, Bae DW, Park SH, Shim CK, Kwak YS, Kim HK (2001) Occurrence and biological control of postharvest decay in onion caused by fungi. Plant Pathol J 17:141–148

Lodewyckz C, Vangronsveld J, Porteous F (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21:583–606

Lottmann J, Heuer H, Smalla K, Berg G (1999) Influence of transgenic T4 lysozyme-producing potato plants on potentially beneficial plant-associated bacteria. FEMS Microbiol Ecol 29:365–377

Lottmann J, Heuer H, de Vries J, Mahn A, During K, Wackernagel W, Smalla K, Berg G (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 33:41–49

Lübeck PS, Hansen M, Sørensen J (2000) Simultaneous detection of the establishment of seed-inoculated *Pseudomonas fluorescens* strain DR54 and native soil bacteria on sugar beet root surfaces using fluorescence antibody and in situ hybridization techniques. FEMS Microbiol Ecol 33:11–19

Martinez C, Michaud M, Bélanger RR, Tweddell RJ (2002) Identification of soils suppressive against *Helminthosporium solani*, the causal agent of potato silver scurf. Soil Biol Biochem 34:1861–1868

Melero-Vara JM, Prados-Ligero AM, Basallote-Ureba MJ (2000) Comparison of physical, chemical and biological methods of controlling garlic white rot. Eur J Plant Pathol 106:581–588

Michereff SJ, Silveira NSS, Reis A, Mariano RLR (1994) Epiphytic bacteria antagonistic to *Curvularia* leaf spot of yam. Microb Ecol 28:101–110

Nagtzaam M, Bollen GJ (1997) Colonization of roots of eggplant and potato by *Talaromyces flavus* from coated seed. Soil Biol Biochem 29:1499–1507

Nautiyal CS, Mehta S, Singh HB (2006) Biological control and plant growth promotion by *Bacillus subtilis* from milk. J Microb Biotechnol 16:184–192

Nielsen MN, Sørensen J, Fels J, Pedersen HC (1998) Secondary metabolite- and endochitinase-dependent antagonism toward plant-pathogenic microfungi of *Pseudomonas fluorescens* isolates from sugar beet rhizosphere. Appl Environ Microbiol 64:3563–3569

Nielsen TH, Christophersen C, Anthoni U, Sörensen J (1999) Structure and production of a new bacterial cyclic depsipeptide; viscosinamide antagonistic against *Pythium ultimum* and *Rhizoctonia solani*. J Appl Microbiol 87:80–90

Okigbo RN (2002) Mycoflora of tuber surface of white yam (*Dioscorea rotundata* Poir) and postharvest control of pathogens with *Bacillus subtilis*. Mycopathologia 156:81–85

Okigbo RN (2005) Biological control of postharvest fungal rot of yam (Dioscorea spp.) with *Bacillus subtilis*. Mycopathology 159:307–314

Okigbo RN, Ikediugwu FEO (2008) Studies on biological control of postharvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride*. J Phytopathol 148:351–355

Osburn RM, Schroth MN, Hancock JG, Hendson M (1982) Dynamics of sugar beet seed colonization by *Pythium ultimum* and *Pseudomonas* species: effects on seed rot and damping-off. Ecol Epidemiol 79:709–716

Pandey P, Kang SC, Gupta CP, Maheshwari DK (2005) Rhizosphere competent *Pseudomonas aeruginosa* GRC1 produces characteristic siderophores and enhances growth of Indian mustard (*Brassica compestris*). Curr Microbiol 51(5):303–309

Pereira de Melo FM, Fiore MF, Beraldo de Moraes LA, Silva-Stenico ME, Scramin S, Teixeira MA, Melo IS (2009) Antifungal compound produced by the cassava endophyte *Bacillus pumilus* maIIIm4a. Sci Agric 66:583–592

Perneel M, Heyrman J, Adiobo A, De Maeyer K, Raaijmakers JM, DeVos P, Hofte M (2007) Characterization of CMR5c and CMR12a, novel fluorescent *Pseudomonas* strains from the cocoyam rhizosphere with biocontrol activity. J Appl Microbiol 103:1007–1020

Proctor FJ, Goodliffe JP, Coursey DG (1981) Post-harvest losses of vegetables and their control in the tropics. In: Spedding CRW (ed) Vegetable productivity, vol 139. MacMillan, London, pp 1–72

Quan CS, Zheng W, Liu Q, Fan SD (2006) Isolation and characterization of a novel *Burkholderia cepacia* with strong antifungal activity against *Rhizoctonia solani*. Appl Microbiol Biotechnol 72:1274–1284

Rauf CA, Ashraf M, Ahmad I (2007) Management of black scurf disease of potato. Pak J Bot 39:1353–1357

Recep K, Fikrettin S, Erkol D, Cafer E (2009) Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. Biol Control 50:194–198

Reddy MS, Rahe JE (1989) Growth effects associated with seed bacterization not correlated with populations of *Bacillus subtilis* inoculant in onion seedling rhizosphere. Soil Biol Biochem 21:373–378

Rosales AM, Thomashow L, Cook RJ, Mew TW (1995) Isolation and identification of antifungal metabolites produced by rice-associated antagonistic *Pseudomonas* spp. Phytopathology 85:1028–1032

Sadfi N, Chérif M, Fliss I, Boudabbous A, Antoun H (2001) Evaluation of bacterial isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. J Plant Pathol 83:101–118

Sadfi N, Cherif M, Hajlaoui MR, Boudabbous A (2002) Biological control of the potato tubers dry rot caused by *Fusarium roseum* var. *sambucinum* under greenhouse, field and storage conditions using *Bacillus* spp. isolates. J Phytopathol 150:640–648

Schisler DA, Slininger PJ, Kleinkopf G, Bothast RJ, Ostrowski RC (2000) Biological control of Fusarium dry rot of potato tubers under commercial storage conditions. Am J Potato Res 77:29–40

Schmiedeknecht G, Bochow H, Junge H (1998) Use of *Bacillus subtilis* as biocontrol agent. II. Biological control of potato diseases. J Plant Dis Prot 105:376–386

Sharma RR, Singh D, Singh R (2009) Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. Biol Control 50:205–221

Stephan H, Freund S, Beck W, Jung G, Meyer JM, Winkelmann G (1993) Ornibactin a new family of siderophores from *Pseudomonas*. Biometals 6:93–100

Sturz AV, Christie BR, Matheson BG, Arsenault WJ, Buchanan NA (1999) Endophytic bacterial communities in the periderm of potato tubers and their potential to improve resistance to soil-borne plant pathogens. Plant Pathol 48:360–369

Swain MR, Ray RC (2008) Optimization of culture conditions and their statistical interpretation for production of indole-3-acetic acid by *Bacillus subtilis* CM5 using cassava (*Manihot esculenta* Crantz) fibrous residue. J Sci Ind Res 67:622–628

Swain MR, Ray RC (2009a) Bio-control and other beneficial activities of *Bacillus subtilis* isolated from cow dung microflora. Microbiol Res 164:121–130

Swain MR, Ray RC (2009b) Oxalic acid production by *Fusarium oxysporum* Schlecht and *Botryodiplodia theobromae* Pat., post-harvest fungal pathogens of yams (*Dioscorea rotundata* L.) and detoxification by *Bacillus subtilis* CM1 isolated from culturable cowdung microflora. Arch Phytopathol Plant Protec 42:666–675

Swain MR, Ray RC, Nautiyal CS (2008) Biocontrol efficacy of *Bacillus subtilis* strains isolated from cow dung against post-harvest yam (*Dioscorea rotundata* L.) pathogens. Curr Microbiol 55(5):407–411

Tambong JT (2000) The Cocoyam root rot disease pathogen, Pythium myriotylum: pathogenicity, isozyme characterization,nature of resistance and biological control. PhD Thesis, Ghent University, Belgium

Tambong JT, Hofte M (2001) Phenazines are involved in biocontrol of *Pythium myriotylum* on cocoyam by *Pseudomonas aeruginosa* PNA1. Eur J Plant Pathol 107:511–521

Thrane C, Nielsen TH, Nielsen MN, Sørensen J, Olsson S (2000) Viscosinamide-producing *Pseudomonas fluorescens* DR54 exerts a biocontrol effect on *Pythium ultimum* in sugar beet rhizosphere. FEMS Microbiol Ecol 33:139–146

Tian SP, Qin GZ, Xu Y (2005) Synergistic effects of combining biocontrol agents with silicon against postharvest diseases of jujube fruit. J Food Prot 68(3):544–550

Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y, Ezaki T, Arakawa M (1992) Proposal of *Burkholderia* gen. nov., transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni, Holmes, 1981) comb. nov. Microbiol Immunol 36:1251–1275