Biocontrol of tomato foot and root rot by Pseudomonas bacteria in stonewool

Validov, S.

Chapter 1

General Introduction

Introduction

Plant diseases have become a permanent threat since human societies started to rely on agriculture as on a major food provider. Back in history, outbreaks of plant diseases resulted in human catastrophes. For example, the Great Potato Famine killed hundreds of thousands of Irish people and forced the emigration to the USA in 1845-1846. Similarly, an epidemic of brown spot rice was the cause of a devastating famine in India in 1943 (Bent 2003). Even nowadays the crop loss due to phytopathogens is still a serious economical problem in agriculture. It is estimated to cause a 15-20 % reduction of the crop yield worldwide (www.apsnet.org). Diseases caused by fungi are the major threat to plants. Out of a million known species, only eight thousand fungi are phytopathogenic (Bent 2003). Fungal pathogens are important not only because they reduce crop yield, but also due to certain compounds they produce during proliferation on/in plants. These compounds, called mycotoxins, are highly poisonous and can adversely affect human and animal health (Pier 1981; Pitt 2000).

1. Pest management

Strategies of pest management were known by humankind in high antiquity. Crop rotation, which breaks life-cycles of soilborne phytopathogens and reduces their build-up, was already mentioned in the Roman literature, and referred to by great civilizations in Africa and Asia. From the medieval time until the 20th century, a three-year rotation was practiced by farmers in Europe: rye or winter wheat, followed by spring oats or barley, and finally letting the soil rest (fallow) during the third stage.

Selection of cultivars which are resistant to certain pathogens happened at farms throughout the history of agriculture. The scientific background for this selection and, the possibility of resistance breeding was discovered in 1905 by the British scientist R.H. Biffen. It was shown that wheat resistance to rust disease was

controlled by a single gene (Biffen 1905). Soon after this, it has been revealed that resistance against many plant diseases is controlled by single genes. Resistance breeding, like many other plant protection methods, has often a temporary nature: it breaks down due to appearance of new strains of the pathogen. Disadvantages of breeding for resistance include a loss of fittness of the plant, and the fact that resistance is not always based on single gene (Robinson 1997).

Another old method is exploitation of solar energy for controlling disease agents in soil and in plant material. This was already used in the ancient India. Crop rotation, resistance breeding and soil solarization are presently still used as simple, effitient and environmentally friendly procedures of plant disease control (Katan et al., 1987).

1.1 Chemical pesticides for crop protection

Crop protection in modern agriculture heavily depends on chemical fungicides. Being extensively used in 1950s -1970s, they seemed to be a final solution against many plant diseases. Disadvantages of chemical pesticides soon became apparent as damage to the environment and a hazard to human health. Moreover, it results in emergence of pesticide-resistant races of the pathogens. Extensive use of pesticides caused a pollution problem in agricultural regions. For example, in 1987 it appeared that surface water in a greenhouse area in The Netherlands must be diluted thirty times before water-flea could survive in it (Working group 1988). Similarly, stable halogen-organic pesticides can be found now in ecosystems far away from the sites where they were applied (Curwin et al., 2005). Due to growing concerns on the negative impact of chemicals, the use of these pesticides is being restricted: more than half of the chemical pesticides used in 1996 were banned in 2003 in the European Union (EU).

The strong reduction of the number of agrochemicals increased the need for alternative plant protection measures. Although genetically engineered pathogen-resistant plants are promising, the European politicians are reluctant or negative

about such products, and genetically modified plants still receive quite a negative public perception.

1.2 Biological control of plant diseases

The use of wild type microbes has become the promising alternative for replacing chemicals or, at least, reducing their use. Over one hundred microbial biocontrol products have been marketed (e.g. Koch, 2001) but their success is variable. This is presumably due to strongly varying conditions in the field since the expression of many biocontrol traits is strongly influenced by biotic (Lee and Cooksey, 2000; Smith et al., 1999) and abiotic (Schnider-Keel et al., 2000; Tomashow and Weller, 1996; Duffy and Défago, 1997, van Rij et al., 2005) conditions. Indeed, it is generally agreed that biocontrol products are more successful under the better controlled greenhouse conditions than in the open field (Paulitz and Bélanger, 2001).

Biosafety of microbial products is a great concern of the society. In fact, many human/animal and plant pathogens can be found among microorganisms which are able to control plant diseases (Bano and Musarrat 2003; Chiarini et al., 2006; Mari et al., 2003). However, the increase of the number of these microorganisms in the environment and even more so their presence in food or forage is highly undesirable. The regulations for microorganisms which can be used as biopesticides vary among countries. According to EU rules, species which have a pathogenic representative cannot be used in agriculture (Anonymous 1998). The United States has less strict requirements. Therefore preparations based on non-pathogenic strains, which belong to species harbouring pathogenic representatives, can be found among commercial biocontrol products (BioFox C, Bio-Save 10, Blue Circle and PSSOL in Table 1).

High efficacy and biosafety are not the only requirements for a biopreparation to be commercialized. Since the majority of biocontrol products must contain live microorganisms (Table 1), methods to preserve them and facilitate their application on the target are important. These methods are known as formulations, e.g.

measures for preservation, delivering to the target and, in some cases, improving the activity of a biocontrol agent. Formulation affects many aspects of a biocontrol organism, including shelf-life and the ability to survive and proliferate in the environment of the target to control the disease. There are many types of formulations, but they all can be divided in seed treatment, wettable powder, liquid and granulation (Jones and Burges 1998). The choice of an appropriate formulation technique depends upon both the biology of the biocontrol organism as well as on peculiarities of the biocontrol process. Fungal strains have been formulated in many different ways (see Table 1) due to robustness and resistance of their spores to drying. Endospore-forming bacteria, such a *Bacillus spp.*, are more suitable for formulation than strains which only exist in the vegetative stage. Dried spores of bacilli can be kept alive for decades at ambient temperature. Spores resist high temperature treatments and can be effectively formulated using spray-drying. Pseudomonads and other Gram-negative bacteria can also be dried; in this case freeze-drying should be applied. These bacteria can be stored as long as bacilli spores as a lyophilizate with no access of oxygen. Freeze-drying is an expensive treatment. Therefore biocontrol products, based on non-sporulating bacteria are frequently marketed as an aqueous suspension of fermenter biomass supplemented by carriers (Table 1).

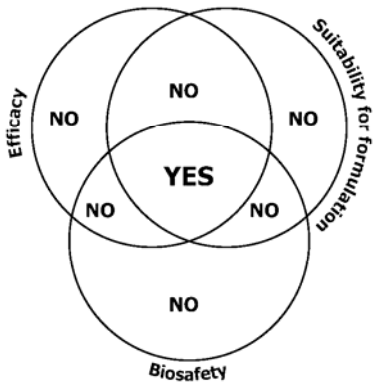

Fig.1 Important criteria for the choice of microorganism meant to be used as a biocontrol preparation

The decision whether a biocontrol strain will be scaled-up and taken in industrial production depends to a great extent on the following characteristics: market size, cost, efficacy, biosafety and the possibility to formulate the biocontrol agent (Fig. 1). Efficacy and suitability for formulation can compensate each other to a certain extent. For example, one of the successful commercial biocontrol products, BlightBan A506 (*Pseudomonas fluorescens*) is supplied as a wettable powder (lyophilizate). Due to its high biocontrol efficacy

against *Erwinia amylovora* (Wilson and Lindow 1993; www.ag.us.nufarm.com), the cost of freeze-drying can be tolerated. An opposite case is a production of biocontrol products based on endo-spore forming bacteria: Kodiak-AT is recommended to apply in combination with chemical fungicides (Jones and Burges 1998), so the biocontrol efficacy of this bacillus is not high. Nevertheless, the low price of the formulation of this strain and the long shelflife of the spores make this commercial product successful (Table 1). Biosafety is a rigorous requirement. Neither high biocontrol efficacy nor ease of formulation are sufficient to allow a pathogenic strain into agricultural practice. Moreover, an application permission of biocontrol products can be revoked, if pathogenisity of their strains have been discovered. An example of it is the fate of biocontrol products based on *Burkholderia cepacia,* which were banned in 2004 after years of successful application (http://www.epa.gov/fedrgstr/EPA-PEST/2004/September/Day-29/p21695.htm).

2. Mechanisms of Biocontrol

The phenomenon of biocontrol by microbes was discovered 70 years ago when studies with suppressive soils were carried out (Baker and Snyder 1965). Some agricultural regions, for instance Salinas Valley (California, USA), the Chateaurenard area (France), the Canary Islands and the Broye Valley (Switzerland) have fields in which agricultural plants do not suffer from the effect of pathogens, although phytopathogenic microorganisms are present in the soil. It was shown that the ability of this kind of soils to suppress pathogens is due to an activity of microorganisms. Elimination of these microorganisms using pasteurization or γ-irradiation makes this soil conducive, i.e. allows the development of the disease (Cook and Rovira, 1976; Scher and Baker, 1980). Moreover suppressivenes can be transferred. If at least 0.1% of a suppressive soil is introduced into a conductive soil, the latter soil can become disease suppressive (Shipton et al., 1973).

An explanation for the way biocontrol microorganisms can inhibit pathogens came from the notion that many soil bacteria can produce antifungal metabolites *in vitro* (Baker and Snyder 1965). Nowadays four mechanisms, which can mediate

biocontrol, are generally recognized: (i) antibiosis, (ii) induction of systemic resistance, (iii) predation and parasitism, and (iv) competition for nutrients and niches.

2.1 Antibiosis

Historically, the antibiosis is the first revealed mechanism of biocontrol and, according to the opinion of some scientists, it is the most efficient one (Haas and Defago 2005). Many rhizosphere bacteria produce secondary metabolites – small organic molecules that inhibit the growth of other microorganisms. The biological role of the production of these compounds is in providing an advantage in colonization of the plant rhizosphere by elimination of competitive microorganisms from the niche (Ligon et al., 2000). Involvement of secondary metabolites in disease suppression was demonstrated by using mutants of *P. fluorescens* CHA0 (Keel et al., 1992) and *P. chlororaphis* PCL1391 (Chin-A-Woeng et al., 1998) impaired in the biosynthesis of 2,4-diacetyl phlorogucinol and phenazine-1-carboxoamide, respectively, which suppressed black root rot of tobacco and foot root rot of tomato, respectively, to a significantly lesser extent than the wild type strains.

2.2. Induction of Systemic Resistance

After interaction with a necrotizing pathogen or with biocontrol bacteria, plants can establish an immunity state that protects them partially or completely from subsequent phytopathogen attacks. Necrotizing pathogens trigger the developing of systemic acquired resistance (SAR) which leads to programmed death of the plant cells near the site of pathogen penetration (van Loon et al., 1998). Proteolytic enzymes and reactive oxygen species that are released from the dying plant cells can kill the pathogens and stop further infection. Salicylic acid accumulates and starts SAR by inducing of production of pathogenesis-related (PR) proteins. Therefore SAR is also called the salicylic acid pathway (Hunt et al., 1996).

Table 1. Examples of commercial biocontrol products for use against soilborne crop diseases [a]

Product biocontrol organism	**Target pathogen**	**Crop**	**Formulation**
BioFox C *Fusarium oxysporum* (non-pathogenic)	*Fusarium oxysporum* *Fusarium molineforme*	Basil, carnation, cyclamen, tomato	Dust or alginate granules
Bio-Fungus *Trichoderma spp.*	*Sclerotinia, Phytophthora, Rhyzoctonia solani, Pythium spp., Fusarium, Verticillum*	Flowers, strawberries, trees, vegetables	Granules, wettable powder, sticks and crumbles
Bio-Save 10 *Pseudomonas syringae*	*Botrytis cinerea, Penicillium spp., Mucor pyroformis, Geotrichum candidum*	Citrus, pome fruit (postharvest disease control)	Wettable powder
BlightBan A506 *Pseudomonas fluorescens*	Frost, *Erwinia amylovora*	Almond, apple, cherry, peach, potato, strawberry,	Wettable powder
Victus *Pseudomonas fluorescens*	*Pseudomonas tolassii*	Mushrooms	Aqueous biomass suspension
Blue Circle *Burkholderia cepacia*	*Fusarium, Pythium*, lesions, spiral, lance, and sting nematodes	Vegetables	Peat carrier or liquid
Ateze *Pseudomonas chlororaphis* 6328	*Pythium, Rhizoctonia, Cylindrocladium, Fusarium*	Pea, ornamentals, cucumber	Powder
Kodiak A-T *Bacillus subtilis*	*Rhizoctonia solani, Fusarium spp., Alternaria spp., Aspergillus spp*	Cotton, legumes	Dry powder (5.5×10^{10} spores/ g) Applied with chemical fungicides
Mycostop *Streptomyces griseoviridis*	*Fusarium spp., Alternaria brassicicola, Phomopsis spp., Botrytis spp., Pythium spp.*	Field, ornamental and vegetable crops	Powder
PSSOL *Pseudomonas solanecearum* (non-pathogenic)	*Pseudomonas solanecearum*	Vegetable	Aqueous biomass suspension
Galltrol-A *Agrobacterium radiobacter*	Crown gall disease, *Agrobacterium tumefaciens*	Fruit, nut and ornamental nursery stock	Petry dishes with pure culture grown on agar (1.2×10^{11} CFU/plate)

a) Jones and Burges 1998, Lugtenberg and Kamilova 2004, http://www.genoeg.net

The plant defence mechanism induced by non-pathogenic bacteria or non-compatible pathogens is known as induced systemic resistance (ISR). ISR was observed in cucumber and tomato against mosaic virus (Raupach et al., 1996), in carnation (Duffy et al., 1983) and in tomato (Kroon, 1990) against *Fusarium oxysporum*, and in *Arabidopsis thaliana* against *P. syringae* pv. *tomato* DC3000 (Zipfel et al., 2004). It was shown that components of bacterial cells such as flagella (Zipfel et al., 2004), lipopolysaccharides (van Loon et al., 1998; Desaki et al., 2006), siderophores (Audenaert et al., 2002), some secondary metabolites such as 2,4 diacetyl phloroglucinol (Iavicoli et al., 2003) and N-acyl-L-homoserine lactone (Schuhegger et al., 2006) and even bacterial cytoplasmic proteins (Kunze et al., 2004) can trigger ISR.

2.3. Predation and parasitism

Some beneficial microorganisms can attack cells of fungal phytopathogens directly by producing lytic enzymes such as chitinases (Carsolio et al., 1994), β(1,3)-glucanases (Lorito et al., 1996), lipases and proteases, which are able to degrade fungal cell wall compounds. This results in destruction of the pathogen, and products of fungal cell degradation can be consumed by the beneficial microorganism. The best known examples of the microorganisms using parasitism and predation as a mechanism are *Trichoderma spp.* (Lorito et al., 1996; Bolwerk, 2005)

2.4. Competition for nutrients and niches

The rhizosphere contains substances such as organic acids, sugars and vitamins, which are exuded from the roots and they are the most important nutrients for the rhizosphere microbes. The mechanism of "competition for nutrients and niches" (CNN) is based on the ability of a biocontrol agent to consume nutrients and to occupy the sites on the roots before the pathogen arrives there. (Lugtenberg and Dekkers, 1999). For the first time CNN was shown to be a sole mechanism for

biocontrol of *Fusarium* wilt of carnation by nonpathogenic *F. oxysporum* strain 618-12. In carnation, treatment with strain 618-12 decreased disease incidence by 80% (Postma and Luttikholt, 1996). Another example, non-pathogenic *F. oxysporum* strain Fo47 suppresses disease only when it is introduced in concentration 10 – 100 higher than the pathogenic *F. oxysporum* f. sp. *radicis-lycopersici* ZUM2407 (Bolwerk et al., 2005). This initial numerical superiority gives Fo47 an advantage to colonize the tomato root faster than the pathogen does and makes this strain capable of disease suppression. Logically, efficient root colonization is an essential characteristic of any biocontrol agent acting through CNN.

So far, CNN is studied for fungus-fungus interaction. One of the strains *F. oxysporum* Fo47 is marketed already in several countries (Paulitz and and Bélanger, 2001).

2.4.1. Exudate consumption

Plants secrete 5% to 21% of all photosynthetically fixed carbon into the rhizosphere as root exudate (Marschner, 1995). Root exudation depends on the substrate in which the plant is growing and it can be altered by microorganisms (Walker et al., 2004). A recent study on root exudation of plants growing on stonewool showed that the root exudates of cucumber, tomato and sweet pepper are similar in composition. Citric, succinic, and malic acids represent the major organic acids, whereas fructose and glucose are the major sugars. Exudation of both organic acids and sugars increases during plant growth. Organic acids represent the major fraction of utilizable carbon, their amounts were considerably higher than those of sugars (Kamilova 2006a).

Efficient consumption of root exudate is an important characteristic of good root colonizers. Mutants of *P. fluorescens* strain WCS365 impaired in organic acid utilisation cannot effectively colonize the plant root (Dekkers 1997; Lugtenberg et al., 2001).

2.4.3. Role of motility and chemotaxis

Another important trait for efficient colonization is motility. It was shown that flagella-less mutants are able to occupy the part of root in the close proximity to the seed (Howie et al., 1987), but they cannot colonize the root tip efficiently (de Weger et al., 1987). Although motility, in relation to root colonization, has been reported to depend on the soil type, the plant and bacterial strains used (Weller and Thomashow, 1994), functional flagella are apparently important for migration of bacteria along the growing root and for reaching the root tip (Lugtenberg et al., 2001).

Plant roots do not produce exudate evenly along their surface. Intracellular junctions are supposed to be the major locations where nutrients are being released from the roots. Another "hot spot" of exudation is the tip of a growing root. Since intracellular junctions are the sites of pathogen penetration into the root tissue (Bolwerk et al., 2005), colonization of these sites as well as of the root tip by beneficial microorganisms is a key event in biocontrol. Bacteria are able to track the exudation sites by chemotaxis. The efficient root colonizer *P. fluorescens* strain WCS365 gives a positive chemotactic response towards tomato root exudates and its major components, such dicarboxylic and tricarboxylic acids, and several amino acids. Strain WCS365 shows no chemotaxis towards exudate sugars (de Weert et al., 2002). Mutants of this strain, which are deficient in sugar utilization, retain their root colonizing ability at the level of wild type strain (Dekkers, 1997). These two observation together with that of the root exudate composition show that (i) sugars are not crucial for *P. fluorescens* strain WCS365 as a carbon source and (ii) chemotaxis drives this excellent colonizer towards several major root exudate compounds. Chemotaxis plays an important role in root colonization: *che*A$^-$ mutants of four *P. fluorescens* strains, which retain their general motility, are impaired in competitive root tip colonization, both in a gnotobiotic system and in non-sterile potting soil (de Weert et al., 2002).

2.4.4 Selection of enhanced root colonizing bacteria

2.4.4.1 Gnotobiotic system to study root colonization

Microorganisms in soil form a complicated network of interactions. Supposedly therefore the soil microflora is quite resistant to introduction of new microorganisms. Non-sterile soil can be used for selection of good colonizers, but fine differences between wild type strain and its mutants cannot be revealed due to background of other microorganisms. Soil also contains nutrients which can influ-

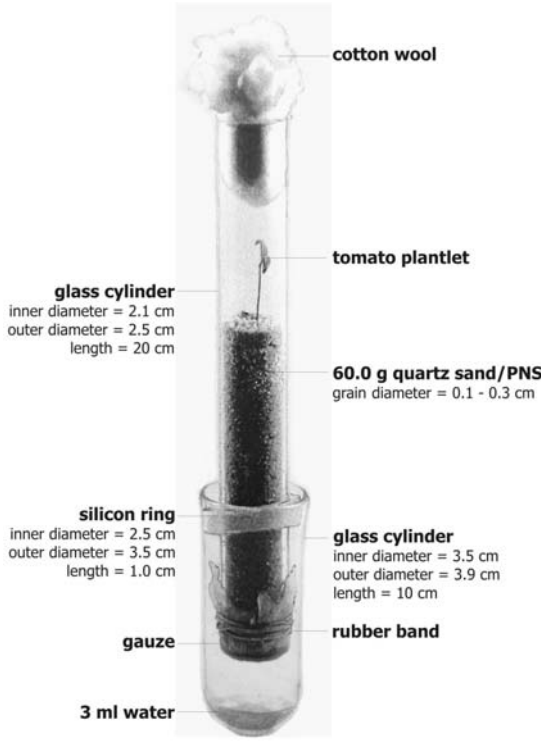

Fig. 2. Gnotobiotic system with growing tomato plant (Simons et al., 1996)

ence colonization of the roots by bacteria. In gnotobiotic system (Fig.2), developed by Simons et al., (1996), bacterized tomato seedlings are planted in a sterile column of quartz sand moistened with plant nutrient solution (PNS, Hoffland et al., 1989). After 7 days of growth in a climate controlled growth chamber, bacteria are isolated from the root. After plating, numbers of bacteria and ratios between wild type and mutant were determined. Using this system a number of traits important for colonization were identified (Dekkers 1997; reviewed by Lugtenberg et al., 2001).

2.4.4.2. Isolation of enhanced root tip colonisers

A criterion for a good root coloniser is that it can efficiently reach the root tip after seed inoculation. An enrichment procedure for rhizoremediating bacteria described by Kuiper et al.(2001a) was developed to isolate efficient naphthalene degrading, root colonising bacteria from soil samples. In this system plant can select good colonizing bacteria. By using this selection procedure, *P. putida* strain PCL1444 was isolated (Kuiper et al., 2001a; Kuiper et al., 2002). By modifying this procedure and subjecting a complete Tn5luxAB mutant bank of WCS365 to this procedure it is possible to select for enhanced competitive root tip colonising mutants. A mutant isolated by using this enrichment appeared to have *mutY* gene disrupted (de Weert et al 2004).

3. *Fusarium oxysporum* as a model pathogen

F. oxysporum (*Fox*) is well represented species among the communities of soilborne fungi, in every soil type worldwide (Burgess 1981). All strains of *Fox* are able to persist on organic matter in soil and to grow in rhizosphere of many plant species (Garret 1970). Many strains of *Fox* are phytopathogenic, they cause rots when penetrating the roots and tracheomycosis, when they invade vascular system of the plant (Fravel et al., 2003). These strains of *Fox* are responsible for yield lost of many economically important crops. *Fox* strains produce variety of mycotoxins,

such diacetoxyscirpenol, HT-2 toxin, deoxynivalenol, trichothecenes, moniliformin, fusarochromanone, fumonisin B1, and wortmannin. These compounds are highly toxic for animals and human. For example, they cause in rats body weight loss, feed refusal, hemorrhage in the stomach and intestines, and, at higher concentrations, death (Mirosha et al., 1989; Abbas et al., 1990).

Being economically important *Fox* species attracts considerable attention of biologists. Genome sequencing project was recently started for this microorganism. A number of scientific teams study pathogenicity and biocontrol of *Fox* strains (Fravel et al., 2003). In our group *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*) strain ZUM2407, a causal agent of tomato foot and root rot, is used to study mechanisms of biocontrol by different microorganisms (Chin-a-Woeng et. al., 1998; Dekker et al., 1999; Bolwerk et al., 2005).

3.1. Taxonomy of *Fusarium oxysporum*

Sexual reproduction has never been observed in *Fox* (Booth 1971). Significant gametic disequilibrium reported among isolates of *Fox* implies that asexual reproduction is an exclusive multiplication strategy in this species (Kistler et al., 1997).

Somatic fusion and heterokaryon formation can occur usually between strains with similar genotypes. This network of strains able to form heterokaryons have been named vegetative compatibility group (VCG).

Fox strains can cause disease to impressive number of plant species. Over than 150 special forms of *Fox* are described (Baayen et al., 2000) as *formae speciales*. Each *forma specialis* includes phytopathogenic strains, which are able to cause disease (wilt or rot) on a unique host or on set of hosts. Since they have the same host, members of a given forma specialis are supposed to be closely related and may have been descended from the common ancestor (Kistler 1997).

However, recent studies revealed ten clonal lineages among strains of *F. oxysporum* f. sp*. cubense* using restriction fragment length polymorphism (RFLP) of anonymous single copy fragment (Koening et al., 1997). These results show that

banana strains of *Fox* f. sp *cubense* could be a closely related to pathogens of another hosts, such as tomato. Considerable genetic diversity within *Fox* f. sp. *cubense* was revealed from the chromosomal polymorphism among the strains random amplified polymorphic DNA and VCGs distribution (O'Donnel et al., 1998). It is generally accepted now that formae speciales which comprise of more than one VCG can have polyphyletic origin (Baayen et al., 2001).

3.2 Monitoring of pathogenic strains of *Fusarium oxysporum*

Detection of pathogen in field, greenhouse or store and monitoring of its development are important parts of pest managements. Classical approach for monitoring of *Fusarium* species is to follow the disease symptoms. The symptoms of different pathogens can be similar, for example, vascular wilt can be caused by *Verticillium dahliae* and *Fox* (Lievens et al., 2003). Therefore this work cannot be carried out without cultivation and identification of the pathogen, which was isolated from lesions.

Many *Fox* strains produce toxins which also can be a target for monitoring (Labuda et al., 2003). This approach is extensively exploited in food and forage production, but it gives more information on the mycotoxins rather than on strains which are producing these compounds.

Microbiological monitoring of filamentous fungi is difficult. Hyphae are continuous structures which are breaking to propagules of different size during the plating. So number of colony forming units (CFU) in the case of fungal material does not reflect real amount of fungal cells (i.e. biomass of the fungus).

Recent progress in molecular biology made possible to follow fungi using specific DNA probes and real-time/quantitative polymerase chain reaction (RT-PCR/qPCR; Schaad and Frederick 2002). Target fragments for qPCR can be obtained using RAPD (Pasquali et al., 2003) or be derived from transposon sequences (Chiocchetti et al., 1999). In addition to estimation of the fungal material amount in the sample, RT-PCR can identify infected plants earlier than symptoms appear (Pasquali et al., 2004).

4. Aims of the thesis

Biological control of phytopathogens gains popularity as an environmentally and end-user friendly approach for crop protection against fungal diseases. It is generally accepted that biocontrol is more reliable under controlled conditions in artificial substrates, such as stonewool, in greenhouses than in open fields. The research, described in this thesis, is aimed at biological control of tomato foot and root rot, caused by *Fusarum oxysporum* f. sp. *radicis-lycopersici* (*Forl*) in stonewool substrates in greenhouses. The aims were the following: (i) To develop an enrichment procedure for the isolation of non-antagonistic bacterial strains which can protect plants against *Forl* (chapter 2); (ii) To use stonewool substrate and isolate and characterize such bacterial strains using this enrichment procedure. One of these isolates is *Pseudomonas putida* PCL1760 (chapter 3); (iii) To unravel the mechanism(s) of action used by *P. putida* strain PCL1760 to control TFRR (chapter 4), (iv) To elucidate the diversity and heterogeneity of *Fusarum oxysporum,* the model phytopatogen used in our biocontrol studies (chapter 5); (v) To quantify *Fusarum oxysporum* biomass in plant tissue as a predictive tool for an ongoing infection (chapter 6) and (vi) To test the efficacy of *P. putida* PCL1760 in the biocontrol of TFRR under industrial conditions in a certified greenhouse under practical conditions using routine and newly developed tools of disease monitoring.