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DIPLOMARBEIT

Response of potato (*Solanum tuberosum* L.) to lipopolysaccharides derived from *Burkholderia phytofirmans* strain PsJN

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Verfasserin / Verfasser:	Valerie Hubalek
Matrikel-Nummer:	9907753
Studienrichtung /Studienzweig (lt. Studienblatt):	Ökologie
Betreuerin / Betreuer:	Ao. Univ.-Prof. Dr. Franz Hadacek Priv.-Doz. DI Dr. Angela Sessitsch

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CONTENTS

Abstract

Endophytic bacteria live inside plants and do not show phytopathogenic properties per definition. It is very likely that all plants are colonized by numerous endophytes. Some of those have been proven to be growth promoting and to be beneficial for their host plant's health.

Since endophytic bacteria and phytopathogens share similar ecological niches, it is possible to apply beneficial bacteria for biological control.

Burkholderia phytofirmans (strain PsJN) is such a beneficial endophyte and it is known for promoting growth and health in *Solanum tuberosum* and other plants. The infection with PsJN can lead to priming, which is the ability of the plant to react faster and / or stronger on encounter with a pathogen.

Bacterial lipopolysaccharides (LPS) are important players in many plant-microbe interactions. LPS are responsible for the high impermeability of the bacterial outer membrane, and thus protects the microorganism from antibiotic substances. LPS belong to the substance class of pathogen associated molecular patterns (PAMPs), which are also known to trigger priming.

Potato late blight, caused by the oomycete *Phytophthora infestans* is a persistent pest that yearly causes the industry a loss in billions.

The aim of this study was to investigate the effects of treatments with PsJN and its LPS on the potato cultivar MF-II. For this purpose plants were infiltrated with either PBS (control solution), strain PsJN or LPS. The plant responses after inoculation with *Phytophthora infestan*s were studied by investigating the levels of the signaling molecules salicylic acid (SA), nitric oxide (NO) and reactive oxygen species (ROS).

The measurements of NO and ROS suggested, that plants infiltrated with PsJN showed stronger activities compared to those treated with LPS. The results of the SA levels indicated, that PsJN and LPS seemed to suppress the accumulation of SA for a certain time period, a mechanism that probably aims to protect the endophyte from the host plant's immune system. This effect was seen longest in plants infiltrated with PsJN. After inoculation with *P.infestans* the SA levels went up again.

To measure the degree of infection with *P. infestan*s, quantitative real-time PCR was performed. This was primarily done to see if priming had occurred in plants treated with PsJN and LPS. Interestingly, it seemed that the oomycete had grown better on leaves infiltrated with LPS. Since the leaves for this assay were collected after

infiltration, then infected with *P. infestan*s, it is likely that the metabolites, necessary for a successful defense, were not produced in the detached leaves.

The main focus of this study however, lied on finding differences in gene transcription in the potato after treatments with LPS or the bacteria respectively. For this purpose DNA microarrays based on a cDNA library of potato plants treated with *P. infestans* were used for the screening. The results showed that more genes were induced in plants treated with PsJN as compared to those infiltrated with LPS. In both treatments only few genes specific for defense were activated.

In conclusion, this study showed, that the potato plants generally showed stronger reactions on treatment with strain PsJN compared to LPS alone. Thus, it is likely that more components in the bacterial cell are necessary to form a successful plant-microbe interaction.

The use of beneficial microorganisms for plant-growth and pest control becomes more and more an important branch in sustainable agriculture. Since this way of plant protection constitutes an environmental and consumer friendly alternative to chemical pesticides and fertilizers, it is essential to put further research into this field.

1 Introduction

A considerable number of studies has already dealt with beneficial plant-microbe interactions that promote plant health and development. Most of these investigations have focused on rhizospheric bacteria (Lindow & Brandl, 2003, Kuiper *et al.*, 2004, Berg *et al.*, 2005b). All plants analyzed so far showed to host a broad range of bacterial endophytes. Numerous reports have shown that various beneficial microorganisms exhibit mechanisms to control plant pathogens (Sturz & Matheson, 1996, Duijff *et al.*, 1997). Furthermore, bacterial endophytes and phytopathogens colonize a similar ecological niche, an important requirement for a successful biocontrol activity (Lindow & Brandl, 2003, Kuiper *et al.*, 2004, Berg *et al.*, 2005a). However, mechanisms involved in biocontrol are insufficiently understood and a better understanding of the molecular aspects of microbe-induced disease suppression is crucial for the development of successful biocontrol strategies.

1.1 *Solanum tuberosum* (potato) and *Phytophthora infestans* (potato late blight)

Potato (*Solanum tuberosum*, fam. Solanaceae) originates from the Andes and is the world's most widely grown tuber crop and the fourth largest food crop after rice, wheat, and corn (Hobhouse, 2001). Potato was introduced to Europe in 1536 and from there potato was disseminated via sea route to other ports throughout the world (Hawkes & Francisco-Ortega, 1993). Once established in Europe, potato soon became an important dietary component. Only few accessions were initially introduced, which led to a lack of genetic diversity and made the crop vulnerable to disease. In 1845, late blight, caused by the oocmycete *Phytophthora infestans*, spread rapidly through western Ireland, resulting in crop failures that led to the Great Irish Famine (Ristaino, 2002). Today potatoes are grown in 125 countries and more than a billion people worldwide consume them daily. Potatoes provide starch, are rich in vitamin C, possess high levels of potassium and are a good source of fiber. Potato grows very easily in almost any soil and can provide more nutritious food faster and on less land than any other food crop (Mullins *et al.*, 2006).

Von Martius was one of the first to propose that a microorganism was the cause of the potato late blight (von Martius, 1842). Anton de Bary finally determined in 1876

that it was the fungus-like *Phytophthora infestans* that evoked the disease (DeBary, 1876). The genus *Phytophthora* is classified under the family Pythiaceae (water moulds), which belongs to the class Oomycetes in the order Peronosporales. In the past the oomycetes were classified under the kingdom fungi due to some fungus-like characteristics (Barr, 1983). Studies of rRNA sequence (Forster *et al.*, 1990), metabolism (Vogel, 1964) and cell wall composition (Bartnicki-Garcia & Wang, 1983) showed that oomycetes are more related to diatoms, chrysophytes and golden-brown algae in the kingdom Straminophila (Baldauf *et al.*, 2000, Kamoun, 2003). One feature of oomycetes that distinguishes them from true fungi is, for example, the compostition of the cell wall. In oomycetes it is prevalently composed of cellulose and β-glucans, and in contrast to fungi contains only little chitin. Oomycetes have a diploid life cycle, whereas true fungi are mostly haploid (Bartnicki-Garcia & Wang, 1983).

The most important characteristic of oomycetes is the ability to form bi-flagellated zoospores (Erwin *et al.*, 1983). *Phytophthora infestans* is a pathogen specialized in causing disease on potato and tomato, although infection of genera other than *Solanum* have been reported (Erwin & Ribeiro, 1996).

1.2 Interactions of bacterial endophytes with plants

1.2.1 Endophytes

In the past only fungi, mostly mycorrhizal fungi (O'Dell & Trappe, 1992) were referred to as endophytes (Carroll, 1988, Clay, 1988). Recently a more comprehensive definition included fungi and bacteria. Endophytes are organisms which at least as a part of their life cycle invade the tissues of living plants, but cause no symptoms of disease (Wilson, 1995). These microorganisms have been isolated from a wide range of plants and can be categorized into obligatory and facultative endophytes. The latter usually colonize the rhizosphere and can enter a host plant through wound sites or through openings at the base of lateral roots, between epidermal cells and at root hairs. For efficient colonization of the plant interior, biofilm formation on the seedling and / or in the rhizosphere is likely to occur (Danhorn & Fuqua, 2007).

Plants present wide and diverse niches for endophytes. They can be found in roots, leaves, tubers, stems, seeds, fruits, ovules and also inside legume nodules (Hallmann *et al.*, 1997, Benhizia *et al.*, 2004). Roots mostly have higher numbers of endophytes compared with above-ground tissues (Rosenblueth *et al.*, 2004). The

most common locations for endophytic bacteria are intercellular spaces and xylem vessels, but rarely in intracellular spaces (Sprent & James, 1995, Reinhold-Hurek & Hurek, 1998).

Endophytic bacteria occur mostly at lower numbers than rhizospheric or pathogenic bacteria (Hallmann *et al.*, 1997, Rosenblueth & Martinez-Romero, 2006). The endophytic lifestyle probably protects the organism better from biotic and abiotic stresses than the rhizosphere environment (Hallmann *et al.*, 1997).

Endophytes can induce growth stimulating effects on their host which can manifest through production of antibiotics and siderophores (Brown, 1974, Burr *et al.*, 1984), synthesis of plant growth promoting substances (Brown, 1972), increased phosphate uptake (Kavimandan & Gaur, 1971) and nitrogen fixation (Trân Van *et al.*, 2000). But in most cases growth stimulation is an indirect effect of reduced disease appearance as many bacterial endophytes are antagonistic to plant pathogens (Brown, 1974, Burr *et al.*, 1978). There are also reports that these organisms can enhance the availability of minerals (Sturz & Nowak, 2000, Sessitsch *et al.*, 2002) for the host plants. Endophytes might also confer biocontrol activities by producing antifungal or antibacterial agents or by the induction of systematic acquired resistance responses in the host (van Loon *et al.*, 1998). Endophytic bacteria from *Solanum tuberosum* have shown antifungal activity (Sessitsch *et al.*, 2004, Berg *et al.*, 2005) and also inhibited growth of *Erwinia* and *Xanthomonas* (Sessitsch *et al.*, 2004). Some endophytes are also able to outcompete invading pathogens.

1.2.2 Burkholderia phytofirmans, strain PsJN

Burkholderia phytofirmans, strain PsJN was originally isolated as a contaminant from *Glomus vesiculiferum*-infected onion roots (Frommel *et al.*, 1991). It is a Gram-negative, rod-shaped, non-sporulating, motile bacterium with a single polar flagellum. Strain PsJN is a highly valuable plant-beneficial bacterium with the ability to promote plant growth (Frommel *et al.*, 1991), and is able to establish rhizospheric and endophytic populations associated with various plants, e.g. potato, grapevines and tomato (Frommel *et al.*, 1991, Compant *et al.*, 2005). Plants living in association with strain PsJN have shown to produce much larger root systems, stronger stems, and more lignin deposits around the vascular system (Nowak, 1998). But most intriguingly, strain PsJN seems to be able to enhance the plant's resistance to potato (Nowak *et al.*, 1995) and tomato pathogens (Sharma, 1998). *B. phytofirmans* strain

PsJN was initially classified as a non-fluorescent *Pseudomonas* species (Frommel *et al.*, 1991) based on biochemical and physiological characteristics. More recent studies however revealed that it belongs to the genus *Burkholderia* (Sessitsch *et al*., 2005). This genus belongs to the β-Proteobacteria and currently contains more than 30 species (Coenye & Vandamme, 2003). Other Burkholderia species are known to interact with plants. *B. cepacia* was originally described as the pathogenic agent of onion soft rot (Burkholder, 1950, Parke & Gurian-Sherman, 2001), but many strains belonging to the *B. cepacia* complex are also able to promote plant health (Parke & Gurian-Sherman, 2001).

Other *Burkholderia* species have been referred to exhibit plant-growth-promoting or biocontrol effects as well (El-Banna & Winkelmann, 1997, Trân Van *et al.*, 2000, Estrada-de los Santos *et al.*, 2001). *B. phytofirmans* promotes the growth of potatoes (Frommel *et al.*, 1991), grapevines (Ait Barka *et al.*, 2000) and vegetables (Nowak *et al.*, 1995), by reducing the level of the growth inhibitory hormone ethylene by secreting a high concentration of 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase (Compant *et al.*, 2005). ACC is the immediate precursor of ethylene (Yang & Hoffman, 1984).

1.3 The plant immune system

Recognition of invertebrate and microbial pathogens and the fast activation of local and systemic defense systems are the key features which are essential for the plant's survival. Unlike animals, plants lack a circulatory system and specific cells, like lymphocytes, to search and destroy dangerous material. But plants do have the functionally equivalent recognition system of basal resistance and an innate immunity which constitutes the first line of defense against invading pathogens (Parker, 2000).

Resistance to a pathogen can be achieved either at the species or at cultivar level. Cultivar level resistance is achieved when specific members of a plant species, but not the whole species, have acquired resistance to a certain pathogen. This form of resistance is often associated with a hypersensitive response (HR) (da Cunha *et al.*, 2006).

Non-host resistance occurs when the complete plant species is resistant to a particular pathogen. Non-host resistance is mostly the outcome of a successful passive defense, such as a pre-formed barrier or toxic chemical. But it can also result

actively from pathogen recognition. Non-host resistance can include a HR, but it is mostly the outcome of a basal defense, which is by definition the defensive response that occurs in the absence of a HR. However, it has to be considered that plant defense responses overlap. An effective resistance induces either a HR or basal defenses, while compatible interactions will result in ineffective basal defenses. For each type of interaction the early biochemical and physiological events are alike. The difference only lies in potency and velocity of the responses. (da Cunha *et al.*, 2006).

1.3.1. Reactive Oxygen Species (ROS)

One of the earliest events in plant cells after encountering a pathogen-derived elicitor is the appearance of quick but large ion fluxes across the plasma membrane (Blumwald *et al.*, 1998). The role of the different ions in plant-pathogen recognition is still unclear. However, there is increasing evidence that the Ca^{2+} homeostasis is the main agent responsible for activation of early plant defense responses (Zimmermann *et al.*, 1997). They occur in plants continuously as by-products in the electron transport chain in chloroplasts, mitochondria and in the plasma membrane cytochrome *b*-mediated electron transfer (Asada, 1999). The accumulation of cytosolic Ca^{2+} eventually leads to the formation of reactive oxygen species (ROS), also referred to as the oxidative burst. Molecules belonging to ROS include the superoxide radical ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$) (Bolwell & Wojtaszek, 1997). Quick production of ROS is also called an oxidative burst and involves the production mainly of $O_2^{\cdot -}$ and H_2O_2 at the site of infection (Apostol *et al.*, 1989).

From the Reactive Oxygen Species formed in plants hydroxyl radical ($OH^{\cdot -}$) is the strongest oxidant, which initiates reactions with organic molecules. Fenton and Haber-Weiss reactions are a significant source of $OH^{\cdot -}$ (Wojtaszek, 1997). $OH^{\cdot -}$ is generated by the catalytic reaction from metal ions (Fe^{2+}, Cu^{2+}) with hydrogen peroxide and oxygen as electron donors (Scandalios, 1993, Streller & Wingsle, 1994, Wojtaszek, 1997).

Superoxide disproportionation:

$2\ ^{\cdot}O_2H \leftrightarrow H_2O_2 + O_2$

$2\ ^{\cdot}O_2H + O_2^{\cdot -} + H^+ \leftrightarrow H_2O_2 + O_2$

$2\ ^{\cdot}O_2^{\cdot -} + 2\ H^+ \leftrightarrow H_2O_2 + O_2$

Fenton reaction:

$H_2O_2 + Fe^{2+} (Cu^{+}) \rightarrow Fe^{3+} (Cu^{2+}) + {}^{\cdot}OH + OH^{-}$

$O_2^{\cdot -} + Fe^{3+} (Cu^{2+}) \rightarrow Fe^{2+} (Cu^{+}) + O_2$

Haber-Weiss reaction:

$H_2O_2 + O_2^{\cdot -} \rightarrow {}^{+\cdot}OH + OH^{-} + O_2$

ROS are important signaling molecules that control processes such as pathogen defense, programmed cell death and stomatal functions (Karpinski *et al.*, 1999, Foyer & Noctor, 2005). From the most common forms of ROS, superoxide and hydrogen peroxide are far less reactive than hydroxyl radicals.

Plants have evolved mechanism to limit ROS formation. The mechanisms for enzymatic ROS scavenging include superoxide dismutase (SOD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), and catalase (CAT) (Willekens *et al.*, 1997). Accumulation of ROS in plant cells can result in the formation of the hypersensitive response (HR) and cell wall cross-linking, as well as in the induction of the expression of defense-related genes (Torres & Dangl, 2005).

1.3.2 Hypersensitive Response (HR)

Hypersensitive response is a defense mechanism where the plant tries to prevent the spread of the pathogen throughout the plant. The hypersensitive response is characterized by localized cell death around the pathogen invading site.

The pathogen is recognized by resistance genes, which activate the defense by changing the membrane potential and ion permeability of the plasma membrane. The resistance genes trigger an increase in extracellular pH and K+ (Orlandi *et al.*, 1992), while eliciting an influx of calcium and hydrogen ions into the cell inducing oxidative burst. This results in cell death and local lesion formation. In the second phase cells undergoing HR activate the reactive oxygen species, such as super oxide anions, hydrogen peroxide, and hydroxyl radicals (Lamb & Dixon, 1997).

The HR induces the secondary resistance response, the systemic acquired response (SAR). SAR involves salicylic acid as a signal molecule and leads to the activation of pathogenesis-related protein (Durrant & Dong, 2004).

1.3.3 Nitric Oxide (NO)

NO is under atmospheric conditions a lipophilic diatomic gas and can easily diffuse into the membrane and cytoplasm (Goretski & Hollocher, 1988). The unpaired electron in NO makes it highly reactive with oxygen, superoxide, thiols and transition metals (Hong *et al.*, 2007). NO serves as an important signal in plants and animals (Beligni & Lamattina, 2001). Generation of the NO burst is a key feature of the plant defense response following pathogen recognition (Delledonne *et al.*, 1998; Durner *et al.*, 1998). Maximal NO accumulation occurres within 4-6 h after

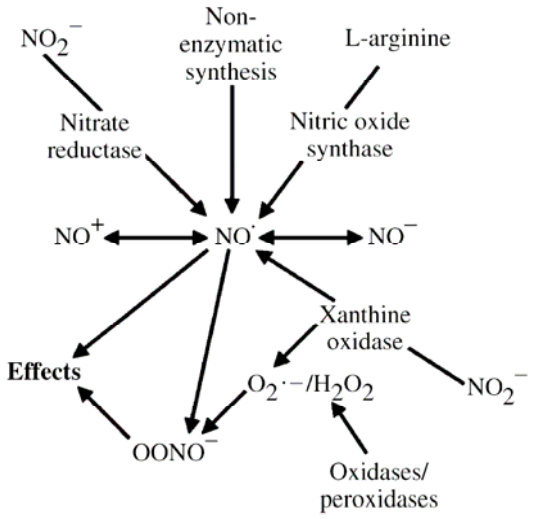

Figure 1: Sources of NO in plants (Neill et al., 2003)

pathogen recognition (Hong et al., 2007). Nitric oxide synthases (NOS), a family of enzymes, are responsible for the generation of NO in animals. The existence of NOS in plants has only been shown in some examples of higher plants (Ninnemann & Maier, 1996). Other potential sources of NO in plants include nitrate reductase (NR), xanthine oxidoreductase or nonenzymatic sources (Figure 1).

NO regulates many stress-inducible and developmental processes in the plant as well. It is involved in root growth, gravitropic bending, flowering, stomatal closure, orientation of pollen tubes, germination, hypoxia, iron availability, adaptation to stresses as well as in cell death (Delledonne, 2005). There is also a considerable overlap and cross talk with ROS signaling. NO functions in combination with ROS to potentiate the hypersensitive cell death and is important for the establishment of disease resistance (Delledonne *et al.*, 1998, Durner *et al.*, 1998, Delledonne *et al.*, 2001). NO might also have an antioxidant function during various stresses (Beligni & Lamattina, 1999).

NO induces the expression of the defense-related genes encoding phenylalanine ammonia lyase (PAL), a marker for phenylpropanoid biosynthesis and pathogenesis-related protein 1 (PR1), the marker for salicylic acid (SA)-mediated signaling (Delledonne *et al.*, 1998; Durner *et al.*, 1998). NO has also been postulated to

function in basal disease resistance triggered by recognition of lipopolysaccharides (LPS), a pathogen-associated molecular pattern (PAMP) (Zeidler *et al.*, 2004).

1.3.4 Salicylic Acid (SA), Jasmonic Acid (JA) and Ethylene (ET)

The response to a pathogen attack is regulated through a complex system of signaling pathways that involve three signaling molecules: salicylic acid (SA), jasmonic acid (JA) and ethylene. The SA and JA signaling pathways work antagonistically. This regulatory communication allows plants to fine-tune the induction of their defense responses. There seem to be two major defense signaling pathways: an SA-dependent and an SA-independent pathway that involves ET and JA. These networks influence each other through a complex system of regulatory interactions (Kunkel & Brooks, 2002). These three signaling molecules are known to play key roles in all different aspects of plant defense. These include response against biotic stresses, such as defense against invertebrate and microbial attack, as well as abiotic stress like wounding and encounter to ozone (Ecker, 1995, Creelman & Mullet, 1997).

SA is required for the rapid activation of certain resistance genes, which induce local defenses that contain the growth of virulent pathogens, and for the development of systemic acquired resistance (SAR). SAR is a state of heightened defense that is activated throughout the plant generated through tissue damage at the site of infection (Ryals *et al.*, 1996). SA is known to activate the expression of pathogenesis related genes from the families PR-2 (β-1,3- glucanases), PR-5 (thaumatin-like proteins), and PR-1 with unknown biochemical properties (Uknes *et al.*, 1992).

JA is not only involved in several defense aspects such as wounding, ozone and pathogen encounter, but in pollen and seed development as well. Genes that encode pathogenesis-related proteins, including *Plant Defensin1.2* (*PDF1.2*), *Hevein-like protein* (*HEL*) and *chitinase B* (*CHIB*), are commonly used to monitor JA-dependent defense responses *Arabidopsis thaliana* (Reymond & Farmer, 1998, Li *et al.*, 2001).

ET is known to activate *CHIB, HEL, PDF1.2* genes as well. It mostly contributes to resistance, but in some cases it can promote disease production (Bent *et al.*, 1992). The JA and ET signaling pathways are also both essential for the development of induced systemic resistance (ISR), another form of systemic resistance that is triggered by the non-pathogenic rhizobacterium *Pseudomonas fluorescens* (van

Wees *et al.*, 1999). JA and ET signaling pathways have also been shown to interact with each other (Kunkel & Brooks, 2002).

1.3.5 Strategies of pathogens to suppress plant defense

Pathogens, on the other hand, have evolved diverse strategies to subvert the plant defense by interfering with core components of plant immunity. Some bacterial toxins target the JA signaling to suppress the expression of defense genes. Other pathogens undermine cell wall-based defenses, whereas some microorganisms are able to enhance their growth by modulating the HR-based programmed cell death (Abramovitch & Martin, 2004).

1.4 The effects of bacterial lipopolysaccharides (LPS) on the plant immune system

1.4.1 The general structure of LPS

Lipopolysaccharides (LPS) are the cell wall components that are associated with the outer membrane of Gram-negative bacteria. LPS is a molecule consisting of a lipid (lipid A), a core oligosaccharide and an O-polysaccharide. The lipid A is located in the outer part of the phospholipid bilayer and is connected to a core oligosaccharide, usually by the sugars 3-deoxy- D-manno-2-octulosonate (KDO) and L-glycero-D-manno heptose (Hep). The core oligosaccharide is made of a short series of sugars such as such as hexoses and hexosamines and connects in the ends with the O-antigen, which consists of repeating oligosaccharide units (Figure 2). Variation is only seen in the structure of the O-antigen part of the molecule and is therefore often used in serotype classification of Gram-negative bacteria (Lüderitz *et al.*, 1971).

Lipopolysaccharides (LPS) are important for the structural and functional integrity of the Gram-negative bacterial outer membrane (Erridge *et al.*, 2002).

LPS play a number of important roles in the interactions of bacteria with eukaryotic hosts. They belong to a group of molecular structures from microorganisms referred to as pathogen-associated molecular patterns (PAMPs). Plant and animal cells have the ability to recognize PAMPs. These general elicitors are not found in the host plant and are very common and conserved structures in various microorganisms. The most relevant PAMPs are flagellin, peptidoglucans (PG), LPS and lipopeptides (LP). (Nürnberger *et al.*, 2004)

In the bacteria, LPS contribute to the membrane permeability properties of the outer membrane, allowing the bacteria to grow and survive in harsh environments. It allows the microorganism to act as a barrier against preformed or induced antimicrobial substances of plant origin. This is supported by testing of bacterial mutants with defects in LPS synthesis, showing increased antibiotic sensitivity (Kingsley *et al.*, 1993, Dow *et al.*, 1995).

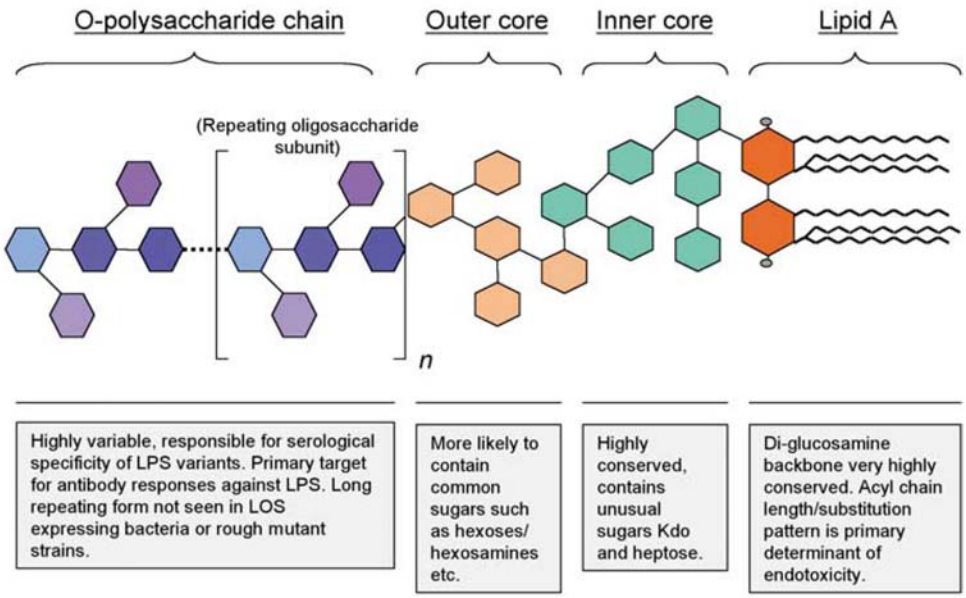

Figure 2: Structure of lipopolysaccharides (Erridge et al., 2002)

1.4.2 LPS and Priming

It has been shown that pre-treatment with LPS from avirulent bacteria can result in priming, thus in an increased capacity to mobilize infection-induced cellular defense responses in plants (Katz *et al.*, 1998, Coventry & Dubery, 2001, Newman *et al.*, 2002, Conrath *et al.*, 2006). These induced responses act to eliminate pathogens or reduce any symptoms due to pathogen attack. These responses include the formation of ROS (Doke *et al.*, 1995, Gerber *et al.*, 2004, Desaki *et al.*, 2006), NO (Zeidler *et al.*, 2004), cell-wall strengthening (Hammerschmidt & Kug, 1982, Schmele & Kauss, 1990), and the expression of various defense-related genes (Ryals *et al.*, 1996). Typical defense or stress-associated genes include glutathione S-transferases, cytochrome P450-type enzymes, and many genes encoding PR proteins, e.g. *PR-1*, which is mediated by NO (Coventry & Dubery, 2001, Zeidler *et*

al., 2004). Chitinases and ß-glucanase were found to be induced in rice by treatment with LPS from *Pseudomonas aeruginosa* (Desaki *et al.*, 2006).

LPS do not always act in direct commence of defense responses, but may increase the degree and/or speed of induction upon following pathogen encounter (Newman *et al.*, 2002).

The most investigated effect of LPS on plants is the ability to prevent the hypersensitive response (HR) by avirulent bacteria (Leach *et al.*, 1983). The part of the LPS molecule responsible for the prevention of HR probably lies in the lipid A-core structure (Graham *et al.*, 1977). At first sight it seems to be paradox that LPS activate basal resistance responses but blocks HR. However, HR is generally related with a decline in the number of bacteria. In contrast to that the prevention of HR does not lead to an increased vulnerability of the plant tissue (Newman *et al.*, 2002). It is probable that the prevention of HR and the triggering of basal defenses are a mechanism to induce resistance without catastrophic damage to the tissue (Newman *et al.*, 2007). There are also observations of LPS binding to the host plant mesophyll cell wall and in that way suppressing the growth of potential pathogens (Graham *et al.*, 1977). This mechanism is termed as Localized Induced Resistance or Response (LIR) (Dow *et al.*, 2000). Depending on a plant's response to LPS, LIR requires several hours to become established.

Infiltration with LPS may also result in induced systemic resistance (ISR), which is caused by non-pathogenic rhizosphere bacteria (Pieterse *et al.*, 1998). A number of reports indicate different effects of LPS from rhizobia on plant responses similar to those seen with LPS from pathogens (Soto *et al.*, 2006).

Much is unknown about the mechanisms of how LPS are recognized by plants and how the different plant responses are activated. Recent evidence leads to the idea that perhaps plants have evolved systems of innate immunity which could be similar to the Toll-like receptor system for lipid A in animals (Medzhitov & Janeway, 2000, Nürnberger & Scheel, 2001, Nürnberger & Brunner, 2002).

The intensity in reaction of the plant also varies between the different LPS types. These differences in LPS-mediated responses might be caused by the sugar composition of the O antigen (Bedini *et al.*, 2005), the conformational structure of the molecule, seen in mammalian cells (Schromm *et al.*, 2000, Bedini *et al.*, 2005), the bacterial origin of the LPS or the recognition of the molecule by the plant (Desender *et al.*, 2006).

2 Aims of this study

Besides breeding plants with resistance to certain diseases, there is also an approach of using plant associated bacteria as agents stimulating plant health. Similar to an immunization this feature is called ‘primed’, by definition the stronger and faster induction of defense responses in plants.

The core of this study was to investigate and compare the effects of strain PsJN and lipopolysaccharides (LPS) derived from this bacterium, on the transcriptome of *Solanum tuberosum* (potato) using a potato cDNA microarray. Furthermore we analyzed the influence of PsJN and LPS on the signal molecules NO, ROS and SA in potato leaves after challenge with *P. infestans*. To detect the degree of infection with *P. infestans* on potato leaves, after infiltration with PsJN and LPS, quantitative real-time PCR was performed.

3 Material and Methods

3.1 Plant material

For the plant experiment the true seed variety MF-II was used. This variety shows resistance to *Phytophthora infestans* race 1. In previous experiments MF-II has shown good response to *Burkholderia phytofirmans* strain PsJN.

The plants were propagated from a mother tube by eye cuttings. To obtain a sufficient number of plants several rounds of internode cuttings were performed. The cuttings were placed in pots containing a mix of ED 63® soil and perlite. The plants for the experiment were surface sterilised by dipping them in sodium hypochloride, followed treatment with sterile water. To improve root growth the cuttings were treated with 0.1-0.2% 4-indol-3-yl-butyric acid (Sigma). The cuttings were then placed in pots (9x9x10cm), which were organised in bowls containing three liters of Flory®3 (4g/L H_2O) (EUFLOR GmbH) as substrate. The plants were placed in a plastic containment (200x 80x 50cm) to guarantee high humidity.

The temperature in the greenhouse was set to 20°C from 0:00-5:00 hrs, to 22°C from 5:00 -21:00 hrs and to 20°C from 21:00-0:00 hrs. The humidity in the containments was approximately 90%; maximum temperature reached 28°C. To regulate the temperature in the containments, the covers were opened frequently and a dark cloth

was placed on the covers to shade the plants when solar irradiation became too high. The plants were grown for one month before the treatments as described below were applied.

3.2 Preparation of the infiltration solution of *Burkholderia phytofirmans* strain PsJN

The day before we started our experiments, a loop of *Burkholderia phytofirmans* strain PsJN (PsJN) cells was transferred from a LB plate to liquid LB media (950ml Milli-Q, 10g bacto-trypton, 5g bacto-yeast extract, 10g NaCl) and grown overnight.

3.3 Preparation of the inoculum of *Phytophthora infestans race T4* (complex race)

The complex race T4 is compatible with the potato variety MF-II.

The single-lesion isolate T4 from potato was used for the inoculations. T4 was collected in 2002 from a commercial field of potato cultivar Laura in Austria. It possesses the complex pathotype 1.2.3.4.6.7.10.11 (Black *et al.*, 1953), the mitochondrial haplotype 1a (according to the nomenclature of (Griffith & Shaw, 1998), and the A1 mating type (J. Avendaño-Corcoles, unpublished results).

To prepare the inoculum 60 g rye grains were imbibed overnight in 200ml Milli-Q® water. The next day the grains were washed several times with Milli-Q water until the fluid was clear. Then 300 ml Milli-Q was added to the rye and boiled one hour at 70-100°C. The grains were filtered out and the liquid was mixed with 20 g saccharose (Sigma), 15 g bacto-agar (GenXpress Service&Vertrieb GmbH) and filled with Milli-Q up to 1000 ml. The medium was autoclaved and poured into petri dishes. A small piece from an agar plate with the mycelia was transferred to a fresh rye medium plate and grown for a week. Four days prior to the experiments, *P.infestans* inoculum was prepared. For that purpose potato tuber slides from cultivar Naglerner Kipfler (no known R-gene) were used. The tubers were washed thoroughly with a brush under clear water, for disinfection immersed into 70% ethanol and breamed to disinfect the tuber from superficial bacteria. The potatoes were cut into slices and placed on a metal frame in a plastic container. The container was then filled with deionized water. *P. infestans* mycelia was cut out of the agar culture into small pieces and placed on the potato slides, with the culture facing up. Water was sprayed on the tubers and *P. infestans* was grown in the incubator at 18°C for four days.

After four days, sporangia of each isolate grown on tuber slices were collected with sterile water and sporangial suspension was adjusted to 2-5 x 10^4 sporangia ml^{-1}. The suspension was chilled at 4°C for two hours prior to use in order to release oospores. Cell numbers were determined using a Bürker-Türk counting chamber. The inoculation of the sporangial suspension (20.000/ml) was done using a hand-held pump sprayer as described by Trognitz (Trognitz, 1998).

3.4 The experiment

For the experiment six containers each with 12 MF-II plants in the same physiological stage were placed in the glasshouse. The plants were placed in two cabinets made of transparent plastic and the relative air humidity was kept above 80% by frequent watering (Figure 3). One hour before infiltrations the plants were irrigated to increase the humidity in order to open the stomata.

Three different infiltrations were carried out: One infiltration with 1xPhosphate Buffered Saline (PBS) (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, in 1000 ml destilled water, pH 7.4) as a control treatment. The second one with PsJN (approximately 5*10^4 cells/ml) and the third infiltration with purified lipopolysaccarides (500µg/ml) extracted from PsJN (a kind gift from Antonio Molinaro, Dipartimento di Chimica Organica e Biochimica, Universita' di Napoli Federico II, Italy).

From each container one box of 12 plants was infiltrated with one of the treatments. The middle lateral leaflet of the third leaf from the bottom was infiltrated with a syringe (using no needle) containing 1 ml of the respective solution (Figure 4).

About 24 hours later, the middle leaflet of the fourth leaf from the bottom was taken and placed immediately into liquid nitrogen. Three leaflets from the same treatment were pooled together. This plant material was used for RNA isolation and subsequent microarray analysis as well as for the measurement of salicylic acid (SA) and jasmonic acid (JA).

At the same time leaflets for the detached assay with *P. infestans* were taken from each plant. The plants from one container were inoculated with the prepared *P. infestans* inoculum about 30 hours after the infiltration with PsJN, LPS or PBS. The other container was sprayed with water and served as the control treatment. Twenty-four hours after the inoculation leaflets were taken from each treatment. Again three leaflets were pooled and placed immediately into liquid nitrogen. These samples were used for the measurement of SA and JA (Table 1).

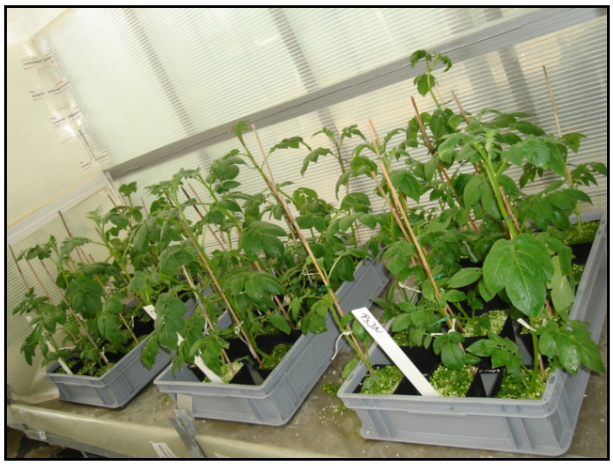

Figure 3: Experimental setup in the glasshouse container with 12 plants in each box.

Table 1: Sampling arrangement

Sampling	Handling	Purpose
24h after infiltration	pooling three leaves from three different plants of the same treatment→ in liquid nitrogen	Microarrays
	Detached leaflet assay	Measurement of ROS and NO inoculation with *P.infestans*→ quantitative real-time PCR
48h after infiltration and 24h after inoculation with *P.infestans*	pooling three leaves from three different plants of the same treatment→ in liquid nitrogen	SA, JA

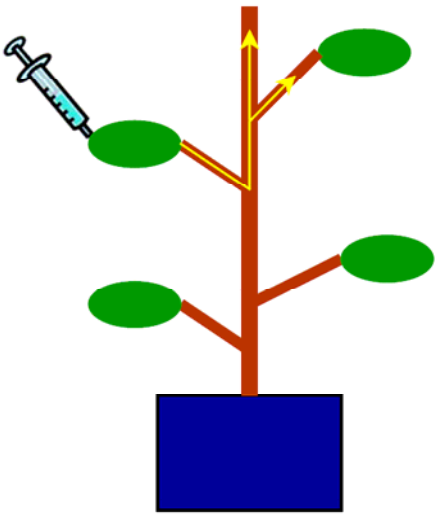

Figure 4: Infiltration with a syringe

3.5 Measurement of Reactive Oxygen Species (ROS)

A reliable and specific method to detect ROS is the use of 2',7'-dihydrodichlor-fluorescein-diacetate (H_2DCFDA) (Invitrogen) (LeBel *et al.*, 1992).

The chemically reduced and acetylated forms of 2′,7′-dichlorofluorescein (DCF) and calcein are nonfluorescent until the acetate groups are removed by intracellular esterases and oxidation takes place within the cell (Figure 5). This process can be detected by measuring the increase in fluorescence.

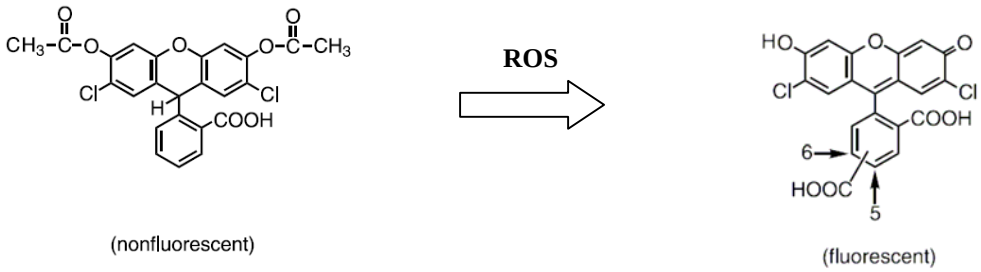

Figure 5: The structure of 2',7'-H_2DCFDA and the deacetylated oxidized product, DCF (Invitrogen product manual)

The ROS measurement was done with leaf stipules 24 hours after infiltration. The leaves were cut carefully to minimize wounding. The working solution of carboxy-H_2DCFDA was prepared by mixing 91 µl of DMSO and 50 µg carboxy-H_2DCFDA (Invitrogen). Twelve leaf stipules each of the three inoculation treatments were placed in deep well plates containing 100 µl of *P. infestans* inoculum in 1xPBS and 10 µM final concentration of H_2DCFDA solution. A negative control was placed in the liquid without *P. infestans* inoculum.

The plates were covered with an optical tape (Biozym) and placed in to the iCycler® IQ Thermocycler (BioRad). Carboxy--H_2DCFDA has excitation/emission maxima of approximately 495/529. Therefore the FAM filter was used. The function of Endpoint measurement was performed every five minutes for a period of an hour (Figure 6).
The mean of the fluorescent value of all 12 samples was calculated and with the T-Test statistical differences between the treatments were determined.

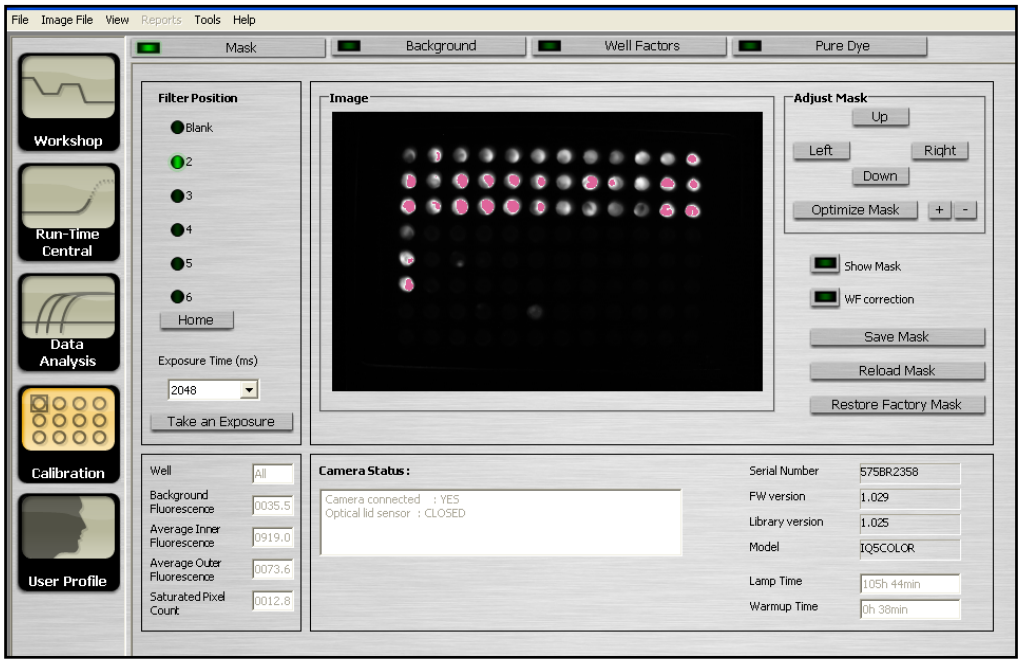

Figure 6: Screenshot example of the Endpoint measurement of ROS in the iCycler after 15 minutes of measurement

3.6 Measurement of Nitric Oxide (NO)

4,5-Diaminofluorescein diacetate (DAF-2 DA) (Calbiochem) is a cell permeable fluorescent detector of nitric oxide in living cells. DAF-2 DA enters cells, where it is hydrolyzed by cytosolic esterases to 4,5-diaminofluorescein (DAF-2), which in turn reacts with NO to triazolofluorescein (DAF-2T), a non-cell permeable fluorescent compound that does not leak into the medium (Kojima *et al.*, 1998) (Figure 7).

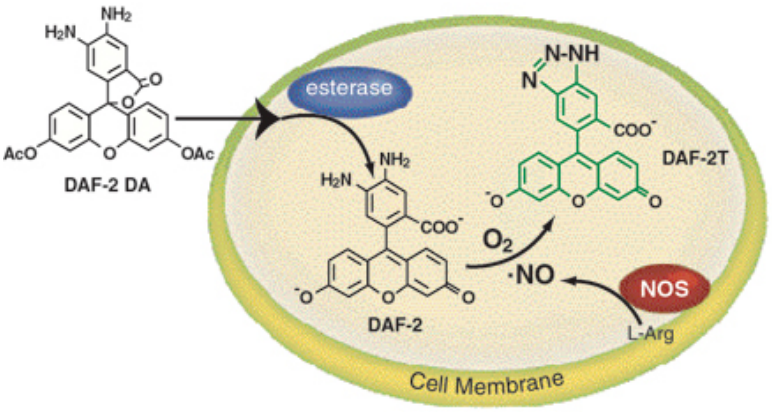

Figure 7: Principle of Nitric Oxide Detection by DAF-2 DA
(source: http://www.emdbiosciences.com/html/cbc/nitric_oxide_probes.htm)

For the measurement of NO 12 leaf stipules for each treatment were placed in the *P. infestans* inoculum in 1xPBS medium. Control leaflets were placed in 1xPBS medium without *P. infestans*. After 30 min the leaves were place in a microtiter plate containing 100 µl loading buffer 5 mM MES (Boehringer Mannheim GmbH), 0.25 mM KCl (Merck), 1 mM $CaCl_2$ (Merck KGaA) and 10 µM DAF-2DA. The fluorescence was measured every 5 minutes in the iCycler (BioRad) using the FAM filter (excitation maximum: 490 nm; emission maximum: 515 nm) (Figure 8). The mean of the fluorescent value of all 12 samples was calculated and with the T-Test statistical differences between the treatments were determined.

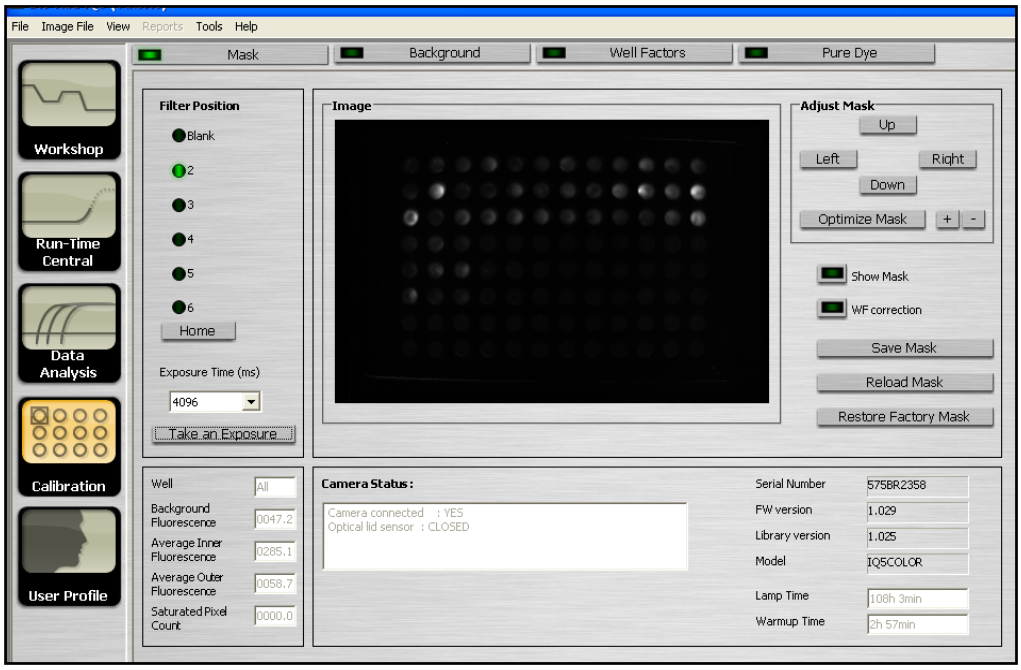

Figure 8: Screenshot example of the Endpoint measurement of NO in the iCycler after one hour of measurement

3.7 Measurement of *Phytopthora infestans* growth after infiltration with PsJN and LPS

Lateral leaflets from each treatment were collected and two leaflets were placed abaxial side up in a Petri dish with wet filter paper. Inoculation of leaflets was carried out by spraying sporangial suspension using a hand-held pump. The sporangial suspension was continuously and gently stirred during inoculation. Incubation was done at 18°C in 12 hour light-periods for five days. After 4 days the leaves were collected and frozen for subsequent isolation of DNA. The DNA of the leaves was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer´s intructions.

The amount of total DNA was calculated using PicoGreen® DNA (Invitrogen).

PicoGreen® is a fluorescent dye that selectively binds dsDNA and has similar characteristics to SYBR® Green. The excitation maximum is at 480 nm and the emission peak is at 520 nm. When bound to double strand DNA, fluorescence enhancement of PicoGreen® is very high. Since the unbound dye has practically no fluorescence, background fluorescence is very low. PicoGreen® is very stable to photobleaching, allowing longer exposure times and flexibility of the assay. Since the mode of binding is not yet fully characterized, potential toxicity is unknown (Ahn *et al.*, 1996).

The measurement of fluorescence was carried out in a 96-well plate with an iCycler® (BioRad). To calculate the amount of DNA 1 µl of the unknown DNA sample was mixed with 49 µl of 1xTE pH 7.5 and filled to a microtiter plate. Lambda DNA (Invitrogen) was diluted to 2 µg/ml and a dilution series was prepared from 500 pg /µl to 3pg/µl in 50µl 1xTE buffer (Table 2). To each of the samples (standard and unknown DNA) 50 µl of 1x PicoGreen (Invitrogen) diluted in 1xTE were added. Samples were then incubated for 5–30 min at room temperature, protected from light. Quantification was performed with iCycler® (BioRad) using Endpoint measurement. The amount of the unknown DNA was calculated by comparing the fluorescence value of the standards to the unknown DNA.

Table 2: dilution series of the standard DNA

µl of (2µg/ml) Lambda DNA stock	µl of 1x TE buffer	µl of diluted PicoGreen reagent	final DNA concentration in PicoGreen assay ng/ml
50	-	50	500
25	25	50	250
12.5	37.5	50	125
6.25	43.75	50	62.5
3.13	46.87	50	31.3
1.56	48.43	50	15.65

The Polymerase Chain Reaction is generally characterized by a sigmoid curve (Figure 9) and the beginning of the exponential phase depends on the starting concentration of the template DNA. The amount of DNA is supposed to redouble after every cycle during the logarithmic phase, and under optimal conditions the PCR efficiency is 100%.

The amplification of DNA is detected with a fluorescent dye that specifically binds to double stranded DNA (dsDNA). In this study SYBR® Green was used for the qPCR assays. According to the manufacturer the fluorescence intensity (extinction: 490 nm, emission: 530 nm) of SYBR® Green increases 10,000 fold once it has bound to dsDNA. In every cycle, after the elongation or annealing step, the fluorescence is measured below the melting temperature of the PCR products to assure that they are still present in their double stranded state (Manit *et al.*, 2005).

Based on standards of known starting concentrations, standard curves are created to determine the number of target molecules in the unknown samples (Figure 10).

In this study quantitative PCR was conducted in 96-well-microtiterplates, using the iCycler5 IQ Thermocycler (BioRad).

To measure the *P. infestans* DNA concentration published primers (O8-3 GAAAGGCATAGAAGGTAGA and O8-4 TAACCGACCAAGTAGTAAA, Judelson & Tooley 2000) were used. The primers are specific and sensitive for detection of *P. infestans*. For the standard curve DNA from *P. infestans* was diluted to 360 pg. From this a 1:10 dilution series was performed until the concentration reached $3.6x10^{-5}$. One µl of the standard was mixed with 11 µl sterile water, 12.5µl SYBRGreen Supermix (BioRad) and 0.75 µl of forward and reverse primer (10 µM). One µl from the DNA of leaves treated with the *P. infestans* were mixed with 12. 5µl SYBRGreen

Supermix(BioRad) and 0.75 µl of forward and reverse primer (10 µM) in a total volume of 25 µl. iCycler settings are described in Table 3. Each standard and sample DNA was run in triplicates. Once the reaction mixtures had been pipetted into the microtiterplate, it was sealed with an optical film. The iQ5 Software (BioRad) calculated the amount of sample DNA in comparison to the standard DNA.

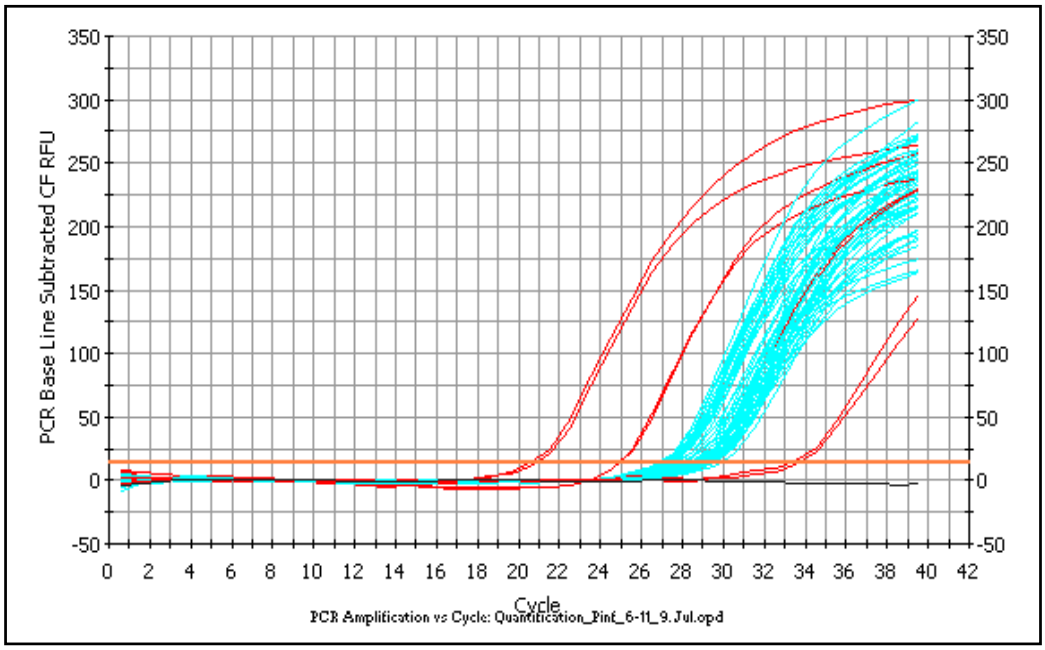

Figure 9: Example of sigmoid curve of qPCR (red: standards, black: negative control, blue: samples)

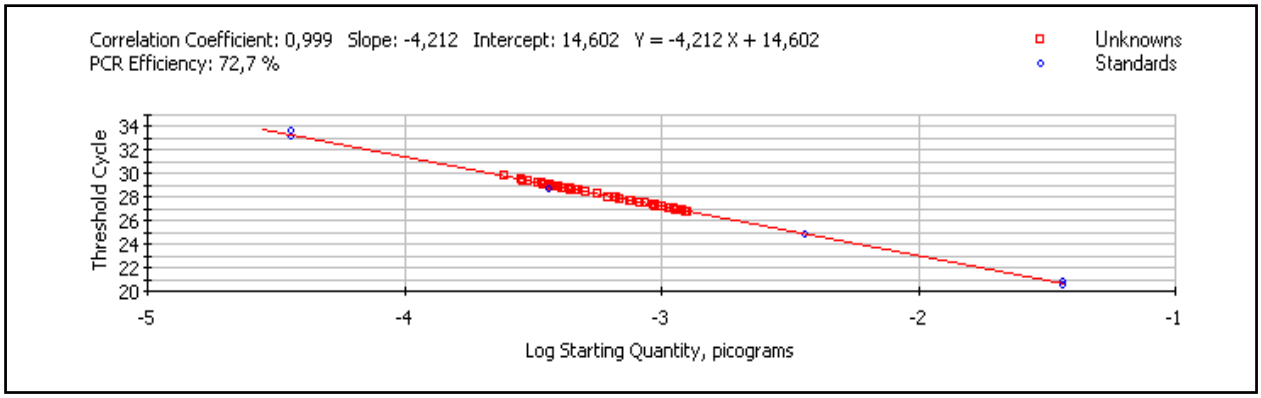

Figure 10: PCR standard curve

Table 3: Description of the iCycler settings

cycle	repeats	step	time	temperature (°C)
1	1	1	3’	95
2	40	1	30’’	95
		2	30’’	50
		3	1’	72
3	1	1	1’	95
4	91	1	30’’	50→ 95 (meltcurve 0.5°C steps)

3.8 Transcriptome analysis of the infiltrated plants

3.8.1 RNA-extraction and cDNA synthesis

The leaf material was ground using a Retsch Mill and the RNA was extracted with Qiagen RNeasy Mini Kit following the manufacturer instruction. The RNA extracts were photometrically quantified using Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, DE, USA).

For labeling of the targets the protocol for template-switching PCR (Petalidis et al. 2003) was applied. In brief, 500 ng of total RNA was mixed with 1 µl 10 pmol 3'SMART CDS primer IIA (5' -AAGCAGTGGTATCAACGCAGAGTAC-T_{30}VN-3'), 1µl 10 pmol of template switching primer [5'd(AAGCAGTGGTATCAACGCA GAGTAC GC) r(GGG)-3' and 1 µl 10 mM dNTP (Invitrogen). The mixture was incubated at 65°C for 5 minutes. On ice 2 µl 5xRT buffer (Invitrogen), 0.5µl DTT, 0.5 µl RNase out and 1 µl SuperScript III (Invitrogen) was added. This reaction mix was left at 50°C for one hour.

For the following long distance PCR 2 µl of the cDNA were used together with 10 µl 10x Advantage 2 PCR Buffer (Takara, Clontech), 2 µl 10 mM dNTPs (Invitrogen), 4 µl 10 pmol PCR primer (5'-AAGCAGTGGTATCAACGCAGAGT-3') and 2 µl Advantage 2 polymerase mix (Takara, Clontech) in a total volume of 100 µl following the cycle conditions described by Petalidis et al. (2003) with 21 cycles (Table 4). Amplicons were cleaned using the QIAquick PCR purification system (Qiagen) and concentrations were measured using the Nanodrop photometer prior to labeling.

Table 4: Description of PCR settings

cycle	repeats	step	time	temperature (°C)
1	1	1	1’	95
2	23	1	5’’	95
		2	5’’	65
		3	6’	68

3.8.2 Microarrays

The microarray was based on a full length cDNA library of *S. tuberosum* var. Yungay, which had been challenged with *P. infestans* complex race for four days. The library was made by VERTIS GmbH (Freising, Germany). The other set of cDNA clones on the array was ordered from the University of Arizona Genomics Institute, and they are also corresponding to stress-related genes. Nine housekeeping genes were included as control spots. The microarray layout is deposited at the NCBI GEO under the following ArrayDesign name: RLP array Version I; ArrayExpress accession: GPL7326 http://www.ncbi.nlm.nih.gov/geo/).

Twenty µl of all amplified clones were lyophilized and then dissolved in 10 µl spotting buffer (3 x SSC and 1.5 M betaine). Probes were spotted in duplicate with an Omnigrid 100 (GeneMachines) equipped with 48 TeleChem pins on CAPS II coated slides (Corning) in a 200 µM spot distance and 40% humidity.

In this study 12 slides were used for hybridization. Always hybridizing one PBS (untreated) sample with either one sample infiltrated with PsJN or with LPS, to analyze the expression pattern of treated plants compared to untreated ones. Each treatment combination was repeated 6 times, performing dye swaps to rule out dye-bias.

Slide processing after spotting

After spotting, the slides were cross-linked at 650 mJ with a Stratagene UV crosslinker. The slides were incubated for 30 min in a freshly prepared solution of 0.25% sodium borohydride (Sigma) in 2xSSC (Sigma) at 42°C followed by two washes with 1xSSC for 5 min at room temperature (Raghavacharie et al. 2003). Then slides were rinsed three times with 0.1xSSC and placed in a coupling jar with 0.1%

N-laurylsacrosine (Sigma) in 5xSSC. The blocking solution was prepared by dissolving 1.75 g of succinic anhydride (Sigma) in 100 ml 1-methyl-2-pyrrolidinone (Sigma), and when the succinic anhydride was dissolved 4.48 ml of 1 M sodium borate buffer (pH 8) was added. The slides were incubated in the blocking solution for 15 min in the dark. Slides were washed 5 times with MilliQ water, placed for 1 min in a 95°C water bath, transferred to 95% ethanol and finally dried with pressured air.

Labeling and Hybridization

Labeling was performed following the instruction of BioPrime® Array CGH Genomic Labeling System (Invitrogen™) with slight modifications.

Fivehundred ng cDNA was brought to a sample volume of 23 µl and transferred into an amber 1.5 ml microcentrifuge tube. 20 µl 2.5X Random Primers Solution was added to each sample and incubated at 95°C for 5 minutes, afterwards immediately cooled on ice for another 5 minutes.

On ice 5 µl 10X dCTP Nucleotide Mix, 1 µl Cy3™ -dCTP or 1 µl Cy5™ -dCTP (GE Healthcare Life Science) and 1 µl Exo-Klenow Fragment was added to the samples. The mixture was vortexed gently and incubated at 37°C for 4 hours. To stop the reaction 5 µl Stop Buffer was added and placed on ice. The mix was applied to the blocked microarray, which was covered by a lifter slip (Erie Scientific). The hybridization was done overnight at 42°C in a hybridization chamber (Camlab).

The next day slides were washed to remove unbound probe. The first (1x SSC with 0.2% SDS) and the second wash buffer (0.1x SSC with 0.2% SDS) were heated in jars to 50°C. When the buffers reached the temperature, the slides were shortly washed in Milli-Q to remove the lifterslips and placed into the first wash buffer for 15 minutes at 50°C in the dark. Afterwards the slides were shaken in the second buffer at room temperature for 10 minutes. Followed by two times of washing in 0.2x SSC at room temperature for 5 minutes. The slides were dried with compressed air and scanned with the LS Series Microarray Laser Scanner (Tecan) using the AGC modus.

Image analysis

The images were processed with GenPixPro6.0 (Axon). Statistical analysis was conducted with the LIMMA package (Wettenhall & Smyth, 2004) of the Bioconductor project (http://www.bioconductor.org) within the R computing environment (www.r-

project.org). Data was normalized within the arrays with the Loess normalization method (Yang *et al.*, 2002). The moderate T statistics in the LIMMA package was used to analyse the data. Spots with a P.value >0.01 were excluded from subsequent analysis. The grouping for functional categories was done according to the MIPS categories.

3.9. Salicylic acid (SA) and Jasmonic acid (JA) measurement

For the SA and JA analysis the infiltrated and inoculated leaf material was used. The extraction was performed according to the slightly modified protocol of (Baldwin *et al.*, 1997).

Extraction solution 1

Acetone (Merck GmbH): 50 mM citric acid (Sigma-Aldrich®) (7:3, v/v) spiked with 100 ng D_5-SA internal standard

Extraction solution 2

Acetone: 50 mM citric acid (7:3, v/v)

The five replicates from every treatment were ground in liquid nitrogen. 150 - 200mg of the plant powder was filled into 2 ml tubes and 1ml extraction solution 1 was added to every sample and placed on ice for one hour. The mixture was centrifuged for 10 minutes at maximum speed. The supernatant was transferred into a new 2 ml tube and placed on a speed vac to evaporate the liquid. The extraction was repeated by adding 1 ml of extraction solution 2 to remaining pellet. This was again placed on ice for one hour and centrifuged for 10 minutes at maximum speed. The supernatant was mixed with the first extraction and evaporated until 1 ml remained in the tube.

The mixture was extracted with 1ml diethyl ether (Merck GmbH) by taking off the upper phase and transferring it into a new tube. This process was done twice and then evaporated completely under vacuum. Afterwards 1 ml diethyl ether was added and loaded onto a 1ml Supelclean LC-NH2 SPE column (Sigma-Aldrich). After the liquid had passed, the column was washed twice with 0.6ml chloroform (Fisher Scientific GmbH) :2-propanol (Fisher Scientific GmbH) (3:1, v/v). The flow-through was discarded. The columns were eluted twice with 0.75 ml diethyl ether: formic acid (Merck GmbH) (98:2, v/v). 100 µl ethanol (Merck GmbH) was added to every sample

and evaporated until dry. The dried extract was dissolved in 60 µl dichlormethane (Merck) and pipetted into a GC-vial. To derivatize 2 µl methanol (Merck GmbH) and 1 µl trimethylsilyldiazomethane (Sigma-Aldrich®) was added. The vials were closed and injected into the gas chromatograph Trace GC 2000 Series (Thermo Quest CE Instruments) where the substances were being separated. This was followed by mass spectrometry (MD 800, Fisons Instruments) for the detection of the analytes.
The settings for injection and measurement were carried out as described by (Montesano *et al.*, 2005).

3.9.1 Quantitative Analysis

The data from the GC/MS was evaluated using the Xcalibur software, a flexible Windows® based data system that also provides instrument control for all Thermo Scientific mass spectrometers and related instruments.
Quantification was performed using an isotope labeled internal standard (ISTD), which acted as a response reference for the components in the sample, which we wanted to quantify (non-ISTD).
Since the ISTD and non-ISTD components are analyzed together, the internal standard quantification approach has the advantage that it corrects for injection and other sample handling errors.
The integral of the graphs of the ISTD and of the non-ISTD of each sample was performed manually. This Manual Area (MA) of ISTD and non-ISTD was used to calculate the nanogramm of SA per gramm of freshweight.

(MA of non-ISTD/ MA of ISTD) x 100 *[amount of ISTD in ng]* **x (1000/ amount of freshweight in mg) = ng SA/ g freshweight**

3 Results and Discussion

3.1 In vivo quantification of Reactive Oxygen Species (ROS) and Nitric Oxide (NO)

3.1.1 Dynamics of reactive oxygen species (ROS) following colonization by strain PsJN and infiltrated with LPS

The quantification of the fluorescence caused by ROS during all endpoint measurements showed strongest ROS production by samples which had been infiltrated with the bacterial endophyte strain PsJN (Figure 11 and Table 5). The comparison to the control group (PBS) revealed a highly significant difference ($P \leq 0.01$) of the signal intensity. The signal intensity of the PsJN-treated plants increased throughout the period in which the measurements were performed, as is shown in Table 5.

The leaves infiltrated with LPS seemed to have a similar fluorescence intensity like those infiltrated with PsJN, and they also differed significantly from the control treatment (Figure 11,Table 5).

Inoculation of plant tissues with pathogens or molecules that are released by microbes including pathogens and non-pathogens may cause an oxidative burst, and production of NO has been demonstrated in many studies (Low & Merida, 1996, Lamb & Dixon, 1997, Bolwell, 1999). Lipopolysaccharides (LPS), a class of bacteria-specific molecules have been shown to cause oxidative stress in many plant species (Desikan *et al.*, 1998, Gerber *et al.*, 2004, Desender *et al.*, 2006, Livaja *et al.*, 2008).

During the accumulation of ROS, programmed cell death (PCD) is induced, to limit pathogen spread from the infection point. ROS production alone without suppressing ROS scavenging would not result in PCD (Delledonne *et al.*, 2001). In our samples ascorbate peroxidase and catalase, genes encoding for ROS detoxifying enzymes, were not significantly active. These enzyme activities are suppressed by salicylic acid (SA) and nitric oxide (NO) (Klessig *et al.*, 2000). ROS production is also known as oxidative burst, it involves the production mainly of $O_2^{\cdot -}$ and H_2O_2 at the site of infection (Apostol *et al.*, 1989). H_2O_2 generation can occur locally and systemically in response to wounding (Orozco-Cardenas & Ryan, 1999) and might function as a signaling molecule mediating the expression of various defense-related genes (Orozco-Cardenas *et al.*, 2001).

ROS can be detected by observing the fluorescent dye fluorescein that is produced from the substrate 2',7'-Dihydrodichlorofluorescein-diacetate (2',7'-H_2DCFDA). This is a non-polar compound able to diffuse into cells where it is hydrolyzed by intracellular esterases to the polar and non-fluorescent derivative 2',7'-dihydrodichloro-fluorescein (H_2DCF), which is trapped within the cells. If intracellular oxidants are present, e.g. H_2O_2, this compound is oxidized to the highly fluorescent compound 2',7'-dichlorofluorescein (DCF) (Keston & Brandt, 1965). The reliability of 2',7'-H_2DCFDA as a method to detect ROS has been shown in several studies from the past decade (LeBel *et al.*, 1992, Gerber *et al.*, 2004). Nonetheless, the method as used in our study represents a novel approach to the quantification of ROS. Instead of merely detecting ROS using fluorescence microscopy, we used the endpoint measurement feature of the iCycler, a device for real-time polymerase chain reaction. With this feature it was possible to quantify and compare the fluorescence response of the different samples in a more accurate way.

The initial use of leaf disks stamped out of entire leaves as experimental samples did not lead to satisfying results due to the relatively large amount of severely damaged tissue from the cut edges. All these samples emitted high levels of fluorescence and no differences related to the colonization by bacteria could be detected. To minimize the effects caused by wounding as much as possible, we used stipules instead of leaf cuttings. Some potato varieties develop such stipules at the central rachis of a leaf, adjacent to the lateral leaflets.

Table 5: Average mean (M) and standard deviation (S) of the ROS fluorescence measurement during the time-frame of 50 minutes.

	PBS+*P.infestans*		**PsJN+*P.infestans***		**LPS+*P.infestans***	
	M	**S**	**M**	**S**	**M**	**S**
10min.	4.21E+03	8.76E+02	5.47E+03	1.49E+03	5.34E+03	1.96E+03
15min.	6.11E+03	1.38E+03	8.61E+03	2.63E+03	8.25E+03	3.10E+03
20min.	9.41E+03	2.08E+03	1.29E+04	4.09E+03	1.21E+04	4.77E+03
25min.	1.20E+04	2.73E+03	1.67E+04	5.39E+03	1.55E+04	6.27E+03
30min.	1.48E+04	3.52E+03	2.11E+04	6.90E+03	1.92E+04	8.11E+03
40min.	1.86E+04	5.48E+03	3.03E+04	1.02E+04	2.67E+04	1.21E+04
50min.	2.67E+04	8.48E+03	4.20E+04	1.44E+04	3.44E+04	1.83E+04

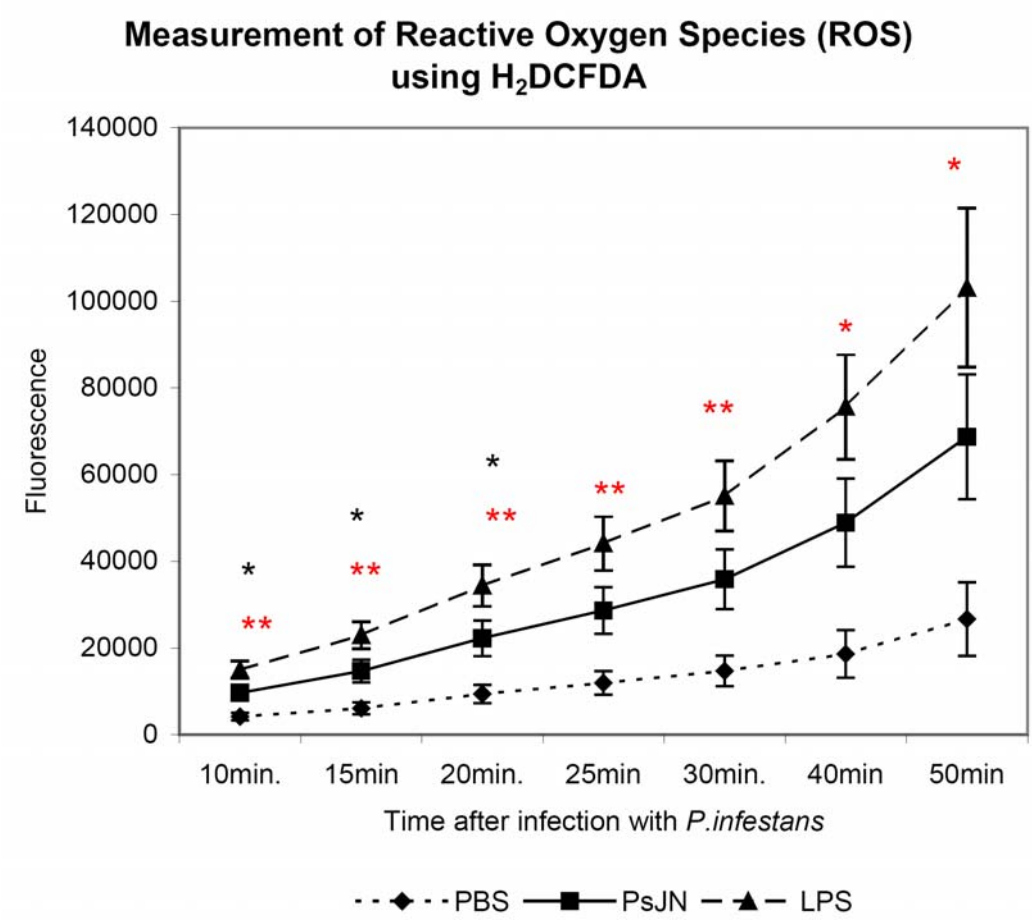

Figure 11: ROS fluorescence during the time-frame of 50 minutes.

** statistically significant difference between PBS and. LPS (P≤ 0.05)*
** statistically significant difference between PBS and PsJN (P≤ 0.05)*
*** high statistically significant difference between PBS and PsJN (P≤ 0.01)*

3.1.2 Dynamics of nitric oxide (NO) following colonization by strain PsJN and infiltration with LPS

NO is a gaseous, free radical which plays an important role in defense responses, plant growth and development (Leshem, 1996, Noritake *et al.*, 1996, Durner *et al.*, 1998).

In the pathogen-activated hypersensitive response, NO and reactive oxygen species (ROS) act as signal molecules (Delledonne, 2005). ROS and NO are also involved in the regulation of SA biosynthesis (Durner *et al.*, 1998) and are key players in the formation of the hypersensitive response (HR), thus essential for the establishment of systemic acquired resistance (SAR) (Alvarez *et al.*, 1998, Nibbe *et al.*, 2002)

NO accumulation again was largest in the samples pre-treated with PsJN, similar to the accumulation of ROS (described above). This effect was highly significantly ($P<0.001$; Figure 12, Table 6) different from the response of the control samples.

PBS-PsJN comparisons were significantly different (P≤ 0.02) at every time point during the period of measurements. A significant difference between LPS and PsJN began to establish 50 minutes after start of the measurements (P< 0.05; Figure 12, Table 6). In contrast, PBS and LPS were not significantly different from each other P>0.05).

In our study, it was interesting that leaves systemically infected with the endophytic, non-pathogenic bacterial strain PsJN always produced a stronger NO accumulation response than infiltrations with LPS. Similar to our findings, Zeidler (2004) described *Burkholderia cepacia* as the strongest inducer of NO, relative to the effect of separate lipopolysaccharides and of other microorganisms.

Table 6: Average mean (M) and standard deviation (S) of the NO fluorescence measurement during the time-frame of 50 minutes.

	PBS+*P.infestans*		**PsJN+*P.infestans***		**LPS+*P.infestans***	
	M	**S**	**M**	**S**	**M**	**S**
10min.	1.88E+02	5.49E+01	4.36E+02	1.52E+02	2.97E+02	7.29E+01
15min.	2.42E+02	1.14E+02	6.52E+02	3.52E+02	3.66E+02	2.43E+02
20min.	3.17E+02	1.83E+02	7.34E+02	4.74E+02	4.38E+02	3.34E+02
25min.	3.44E+02	2.32E+02	8.39E+02	5.42E+02	5.08E+02	4.23E+02
30min.	3.68E+02	2.49E+02	8.84E+02	5.95E+02	5.54E+02	4.74E+02
40min.	4.02E+02	2.52E+02	9.55E+02	6.36E+02	5.43E+02	3.77E+02
50min.	4.45E+02	2.74E+02	1.01E+03	6.79E+02	4.96E+02	2.57E+02

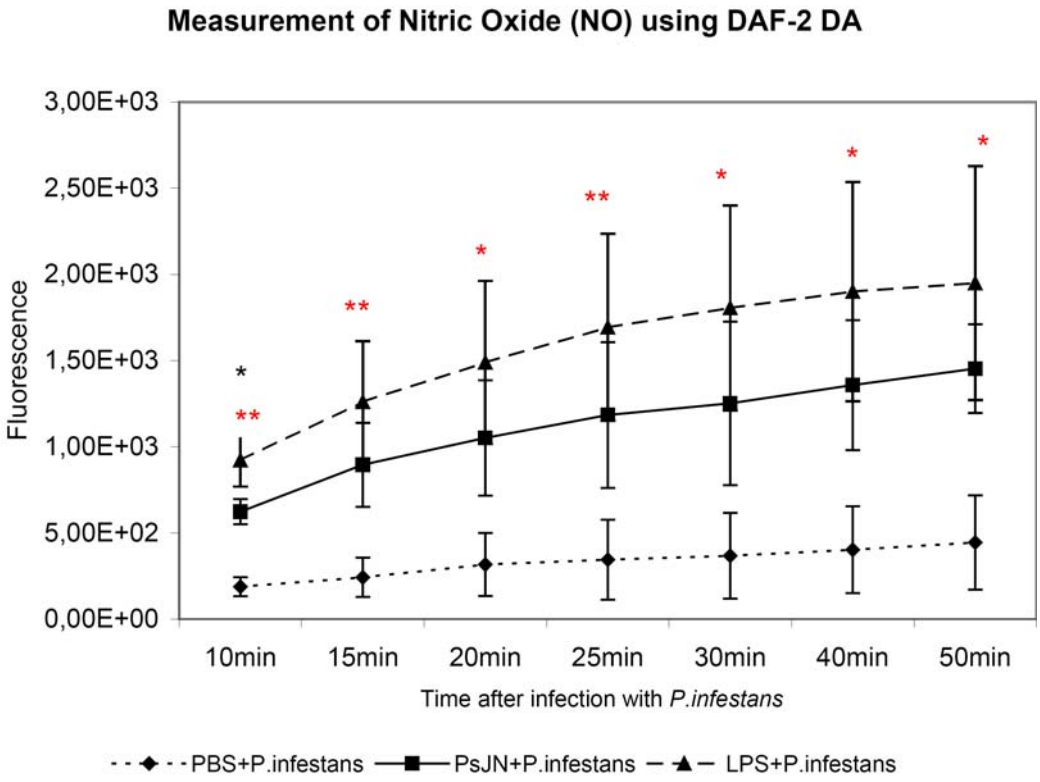

Figure 12: NO fluorescence during the time-frame of 50 minutes.

** statistically significant difference between PBS and. LPS (P≤ 0.05)*
** statistically significant difference between PBS and PsJN (P≤ 0.05)*
*** high statistically significant difference between PBS and PsJN (P≤ 0.01)*

3.2 Measurement of salicylic acid (SA) and jasmonic acid (JA) using gas chromatography-mass spectrometry

SA and JA levels were measured using gas chromatography coupled with mass spectrometry. JA levels were below the detection limit and could not be quantified.

SA levels of plants infiltrated with PBS remained the same throughout the time of the experiment (Figure 13). Interestingly, plants pretreated with PsJN and LPS showed lower SA levels than the control samples on the first day, which indicates the possibility that infiltration with *B. phytofirmans* generally leads to a lowering of SA levels. This effect seemed to be time-limited, because two days after infiltration the SA levels rose, particularly after infection with *P.infestans*. After two days of infiltration LPS pretreated plants did not show significant differences in SA levels, neither in samples infected by *P.infestans* nor in non-infected ones. Whereas PsJN-pretreated plants had significantly altered SA levels in the water inoculated

treatments compared to the control samples (Figure 13) but in the *P.infestans* inoculated treatments the SA level rose up to the level of the control samples.

Treatment with LPS showed only little differences compared to the basal level after two days, which demonstrates that PsJN had a longer-lasting effect.

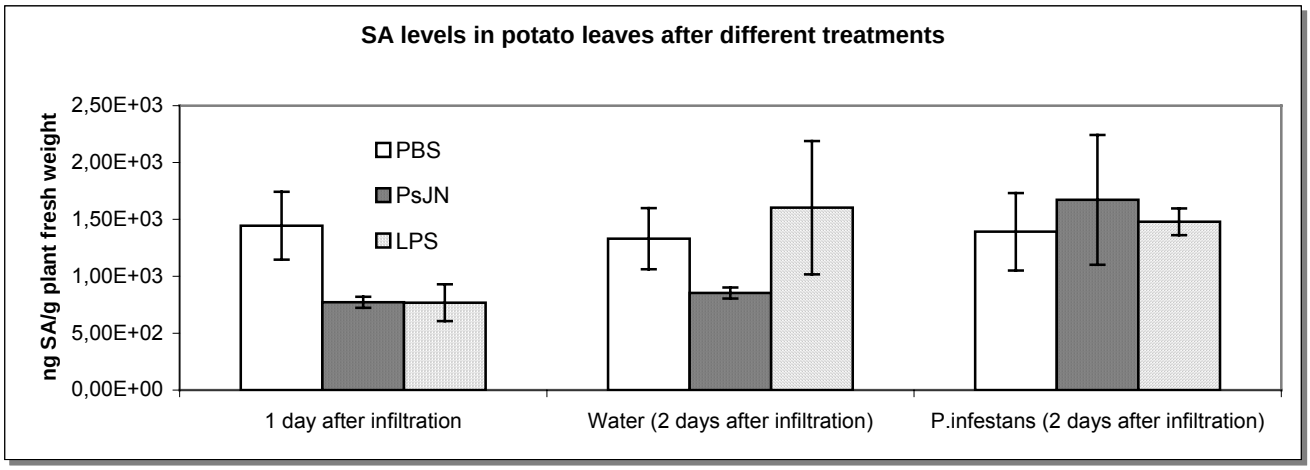

Figure 13: SA levels in potato leaves after the treatments with PBS, PsJN and LPS before and after inoculation with P.infestans.

3.2.1 SA basal level in Solanum tuberosum

Unlike tobacco, Arabidopsis and cucumber, potato and rice have a high SA basal level (Yu *et al.*, 1997, Dong, 1998, Halim *et al.*, 2006). Since increased SA levels in other model plants are associated with enhanced disease resistance (Yalpani *et al.*, 1993, Bowling *et al.*, 1994, Weymann *et al.*, 1995) it is likely that the high SA level found in healthy *Solanum tuberosum* plays a certain role in their defense mechanisms as well (Yu *et al.*, 1997).

The plants in this study which were infiltrated with the control solution PBS showed a constantly high SA level, even during infection with *P.infestans*. SA levels in plants treated with PsJN and LPS were suppressed before pathogen encounter, but after infection, SA accumulated again to the status of the basal level (Figure 13). Thus SA does probably play a role in defense, whereas the level itself is of less importance.

This finding is supported by Yu *et. al* (1997), who showed that a reduction of the SA levels in potato and rice did not cause an increased susceptibility to pathogen infection (Yu *et al.*, 1997). Thus, the high basal SA level in unchallenged potato plants does not seem to induce constitutive resistance to *P. infestans.* Similar findings were suggested by several research groups (Dong, 1998, Roetschi *et al.*, 2001, Smart *et al.*, 2003). Since exogenous applied SA also fails to induce disease

resistance in *S. tuberosum*, it seems that potato has a rather poor SA signal perception and/or transduction mechanism (Yu *et al.*, 1997, Coquoz *et al.*, 1998).
Yu *et al.* also showed that SA plays an important role in SAR formation induced by the elicitor arachidonic acid. Thus, SAR-inducing elicitors such as pathogens or chemicals may activate a rate-determining step in SA signal perception and/or transduction in potato rather than stimulating production of the SA signal, as it occurs in other model plants. It is likely that some aspects of SAR development in tobacco and Arabidopsis differ from those in *S. tuberosum* (Yu *et al.*, 1997).
It is possible that stimulation of SA biosynthesis and processing in tobacco, Arabidopsis and potato are mediated by different signal transduction mechanisms activated by SAR-inducing pathogens or chemicals. Another possibility could be that SAR-activating pathogens or chemicals might activate a shared signal transduction pathway with different consequences on SA biosynthesis and processing in the different plant groups (Yu *et al.*, 1997).

3.2.2 LPS and SA

Plants recognize PAMPs of microorganisms, of a pathogenic or non-pathogenic nature, via pattern recognition receptors, which initiate signal transduction cascades leading to the activation of defense responses (Altenbach & Robatzek, 2007). Little is known about the role of SA in PAMP-triggered immunity, even though its importance for defense signaling has been proved in a previous study with the elicitor arachidonic acid (Yu *et al.*, 1997).
In the case of treatment with LPS, Newman *et al.* (2001, 2002) showed similar results to our finding, namely that LPS alone do not induce SA accumulation. The SA levels were even fivefold less than in plants not pretreated (Newman *et al.*, 2001, Newman *et al.*, 2002), which is in accordance with our findings, showing that the SA produced in LPS treated plants (on day one) were only half as much compared to untreated plants. In the course of time SA levels of LPS-treated and untreated plants approximated.

3.2.3 The role of SA in beneficial plant-microbe interactions

There are local and systemic defense responses that can be similarly triggered by beneficial as well as by pathogenic microorganisms. In this signaling network the plant hormones salicylic acid, jasmonic acid, and ethylene are the key players (Van

Wees *et al.*, 2008). The pathways of these plant hormones cross-communicate and allow the plant to fine-tune its defense response depending on the encountered microbe (Koornneef & Pieterse, 2008). Systemic acquired resistance (SAR) is dependent on SA signaling and is triggered upon infection by necrosis-inducing pathogens (Durrant & Dong, 2004). Induced systemic resistance (ISR) is induced by beneficial rhizobacteria, such as *Pseudomonas fluorescens* WCS417 and requires components of the jasmonic acid and ethylene signaling pathway (Pieterse *et al.*, 1998).

It is known, however, that some beneficial bacteria are able to suppress SA accumulation to avoid the host defense mechanisms, to be able to establish a chronic infection within the plant (Soto *et al.*, 2006). This could be a possible explanation of why the SA levels were low in samples infiltrated with PsJN before inoculation with *P.infestans*.

3.3 Determination of P. infestans biomass on systemic leaves

The amounts of *P. infestans* amplicons obtained from samples pre-infiltrated with LPS compared to the control PBS (Figure 14) were different ($P < 0.01$), whereas no significant differences in the quantities of *P. infestans* DNA were obtained from samples pre-treated with PsJN and LPS ($P > 0.1$). Unexpectedly, this oomycete pathogen appeared to grow better on the plants infiltrated with LPS (Figure 14). We used detached leaflets inoculated with a sporangial suspension of a complex race of *P. infestans* (isolate T4) and incubated during 4 days. Then, DNA was extracted from the leaflets and subjected to the qPCR assay using the Judelson (2000) primers specific for *P. infestans*. As a control, we used DNA from pathogen-free potato MF-II leaflets. No product was amplified from control samples using the *P. infestans* primer proving that this primer worked highly selectively.

For the assessment of the degree of late blight disease caused by *P. infestans,* visual examination or re-isolation from infected plant tissues can be used, although visual examination may not be feasible to reliably quantify the amount of pathogen present. However, with the availability of *P. infestans*-specific primers, real-time PCR has been an ideal technique to detect and quantify this plant pathogen in host tissues, due to its high sensitivity and reproducibility (Schena et al., 2004).

Other studies investigated the effects of endophytes on the disease susceptibility of plants. Melnick *et al.* (2008), for example, detected enhanced disease resistance in

cacao against *Phytophthora capsici* after infiltration with a specific strain of *Bacillus cereus*.

The time-frame of appearance and endurance of induced disease resistance were quite different, depending on the pathosystem. Frequently, endophytes were shown to induce rapid suppression of disease, beginning 3-7 days after infiltration, however, several reports recorded induced plant resistance only after 17-26 days following infiltration with an endophyte (Wilhelm *et al.*, 1998, Ait Barka *et al.*, 2002, Bargabus *et al.*, 2002, Melnick *et al.*, 2008).

In potato, both detached-leaflet and whole-plant assays are routinely used to evaluate resistance to *P. infestans* (Brouwer *et al.*, 2004). Disease assays with detached leaflets are a useful tool to help control environmental conditions and allow efficient testing of large numbers of genotypes (Wastie, 1991). Although the detached leaflet-assay has in many cases proven to produce reliable and reproducible results (Lebreton *et al.*, 1999, Vleeshouwers *et al.*, 1999), one cannot measure all components of plant resistance as expressed in the field (Wastie, 1991, Collins, 1999). More importantly, a leaf detached from the rest of the plant is not supplied by metabolites necessary for priming and subsequent defense responses. Therefore it is likely that the result obtained in this assay is not representative of the whole plant.

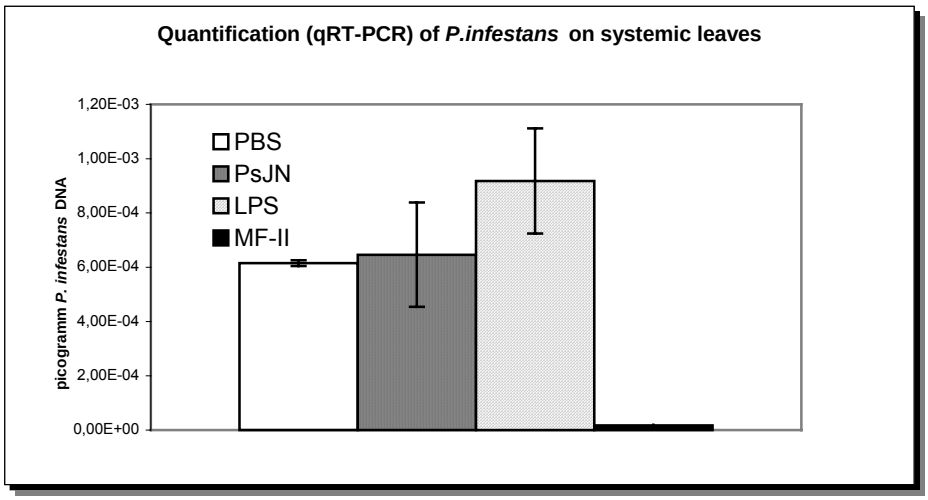

Figure 14: Quantitative real-time PCR of P.infestans on systemic leaves.

3.4 Microarray-based gene expression analysis

DNA microarrays are a useful tool for identifying potential genes related to plant-endophyte interactions. DNA microarray techniques are especially applicable to monitor gene expression changes in plants during plant-endophyte interactions, due to their high throughput, their relative simplicity and broad sampling capacity (Kazan *et al.*, 2001). The most attractive feature of DNA microarray techniques is the possibility to examine the responses of thousands of genes simultaneously (Wan *et al.*, 2002). We studied the gene expression in leaves due to infiltration with PBS, LPS and PsJN after 24 hours.

In total, 5,324 probes on the array that correspond to about 4,000 individual genes were analyzed. Only genes significantly ($P \leq 0.01$) up- or down-regulated (Appendix, Table 7), as determined by two-way t-tests using results from two replicate hybridizations with reciprocal labeling of the samples (dye swap), were considered relevant. For genes that were represented by more than one probe on the microarray, the results of all probes were considered and one representative was chosen when all redundant probes showed comparable results. Genes with two or more probes showing greatly differing hybridization signals were excluded.

With this significance threshold, samples infiltrated with LPS showed 53 significantly up-regulated and 132 significantly down-regulated individual genes. PsJN treatment resulted in 172 up-regulated and 227 down-regulated genes. Thus, samples infiltrated with the bacterium *Burkholderia phytofirmans* strain PsJN showed more statistically significant up-regulated and down-regulated genes than samples infiltrated with LPS. These results imply that LPS alone cannot evoke the same power of response in the plant as the whole bacterium, since there are probably more factors responsible for these mechanisms.

According to the classification by their function, genes regulated after LPS treatment are distributed across specific functional categories issued by the Munich Information Center for Protein Sequences (MIPS) (http://mips.gsf.de/cgi-bin/proj/thal/search_gene?code=At1g10000) (Figure 15, 16). The highest up-regulated categories in LPS included: “unclassified proteins” (18.9%), “metabolism” (13.2%) and “energy” (13.2%), whereas down-regulated genes were classified as “unclassified proteins” (34.1%), “metabolism” (20.0%) and “protein synthesis” (8.2%) (Figure 15, 16).

Accordingly, the most up-regulated genes after PsJN treatment were in the MIPS categories “unclassified proteins” (18.9%), “metabolism“(15.4%) and “protein synthesis” (17.8%). Categories with the down-regulated genes were “unclassified proteins” (32.9%), “metabolism“(17.1%), “protein with binding function or cofactor requirement” (10.1%) (Figure 15, 16).

Categories which were not up-regulated in LPS treatments included: “transcription”, “cellular communication/signal transduction mechanism” and “cell fate or subcellular location”, which was also not found in the down-regulated genes.

Except for wound responsive protein (AM909212), where infiltration with LPS induced down-regulation and PsJN up-regulation, the comparison between significantly regulated genes in both treatments showed almost no difference, meaning that expression was in both either up-regulated or down-regulated.

The highest up-regulated gene in LPS infiltrated samples was the mitochondrial carnitine/acylcarnitine carrier-like protein, which was almost 17-fold up-regulated compared to the control plants. This protein transports carnitine or acylcarnitine from the cytosol to the mitochondrial matrix as an alternative or a complement to the succinate-producing glyoxylate cycle reference. Recently it was demonstrated to be involved in the transition from the embryonic stage to the juvenile autotrophic stage of *Arabidopsis* plants (Lawand *et al.*, 2002).

Other highly up-regulated genes with a fold change ratio (FCR) ≥2.0 in LPS treated plants include: calcium ion binding protein, heat shock protein HSP70, cysteine protease family protein and 60S ribosomal protein L18. Altered ion fluxes, including receptor-mediated changes in levels of free calcium in the cytoplasm, constitute the earliest events during an immune response and stand at the beginning of a signaling cascade (Nürnberger & Scheel, 2001). Heat-shock proteins play important roles as molecular chaperones that ensure the correct folding of proteins and are known to be involved in stress responses. They can be induced either by high temperature or by biotic or abiotic stress (Gjetting *et al.*, 2004, Waller *et al.*, 2008). HSP70 is activated by a high range of stress conditions and signals such as plant hormones, osmotic stress, chemical stress, pathogen encounter, heat and cold (Cheong *et al.*, 2002, Wang *et al.*, 2004). Cysteine proteases are induced in plant systems undergoing programmed cell death (PCD) (Solomon *et al.*, 1999), and are induced in certain forms of cell aging (Drake *et al.*, 1996).

PsJN infiltrated plants showed the highest up-regulation for flavonol-3-O-glycoside-7-O-glucosyltransferase 1, with a FCR of 3.3, followed by phosphorylase family protein with a FCR of 3.1. Glycosyltransferases are probably involved in plant defense and stress tolerance (Vogt & Jones, 2000). They are responsible for converting reactive and toxic aglycones into stable and non-reactive storage forms, thus limiting their interaction with other cellular components. Glucosyltransferases are a type of glycosyltransferase which enable transfer of glucose (Jones & Vogt, 2001).

Other genes with a FCR ≥2.0 in PsJN treated plants are: 2-oxoglutarate-dependent dioxygenase, GDSL-motif lipase/hydrolase family protein, Sterol desaturase family protein, plant lipid transfer protein, hydrophobic protein, plant defensin-fusion protein, proteinase inhibitor I and wound-induced aspartate proteinase CDI inhibitor.

Several members of the plant defensin family inhibit growth of a broad range of fungi and other microorganisms at micromolar concentrations (Broekaert *et al.*, 1995).

For our investigations proteinase inhibitors are also interesting, as they play important roles in defense as well (Doares *et al.*, 1995).

Kinase/protein binding showed a 12-fold down-regulation compared with the control group in plants infiltrated with LPS. Xyloglucan endotransglucosylase-hydrolase, a gene probably involved in plant growth promotion (Wang *et al.*, 2005), was 4.6-fold down-regulated. Genes with a down-regulation FCR ≥2.0 included: lactoylglutathione lyase, trypsin-like protease, a proteine involved in cytochrome oxidase assembly, pectinesterase inhibitor and nonspecific lipid transfer protein 1.

Xyloglucan endotransglucosylase-hydrolase showed the highest down-regulation in plants infiltrated with *B. phytofirmans* as well, with a FCR of 10.9. Leucine-rich repeat resistance protein-like protein was 4.3 fold down-regulated, compared to the control plants. Genes with a down-regulation FCR ≥2.0 in the PsJN group included: COP9 signalosome subunit, calcium- and calmodulin-dependent protein kinase, chitinase class IV, protein kinase family protein, clathrin adaptor complex small chain family protein, Curculin-like (mannose-binding) lectin family protein, nonspecific lipid transfer protein 1 and heavy-metal-associated domain-containing protein.

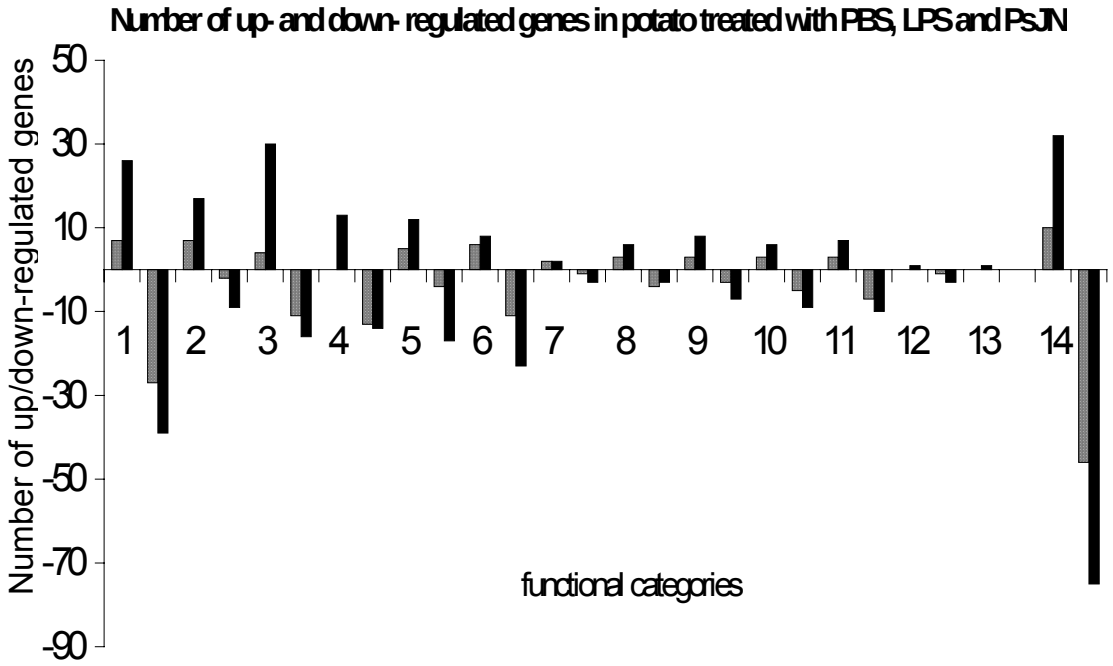

*Fig.15: Number of up- and downregulated genes in the functional categories. **1**-Metabolism, **2**-Energy, **3**-Protein synthesis, **4**-Transcription, **5**-Protein fate (folding, modification, destination), **6**-Protein with binding function or cofactor requirement (structural or catalytic), **7**-Regulation of metabolism and protein function, **8**-Subcellular localization, **9**-Cell rescue and defense, **10**-Interaction with the environment, **11**-Cellular transport, transport facilities and transport routes. Grey bars- LPS, black bars- PsJN*

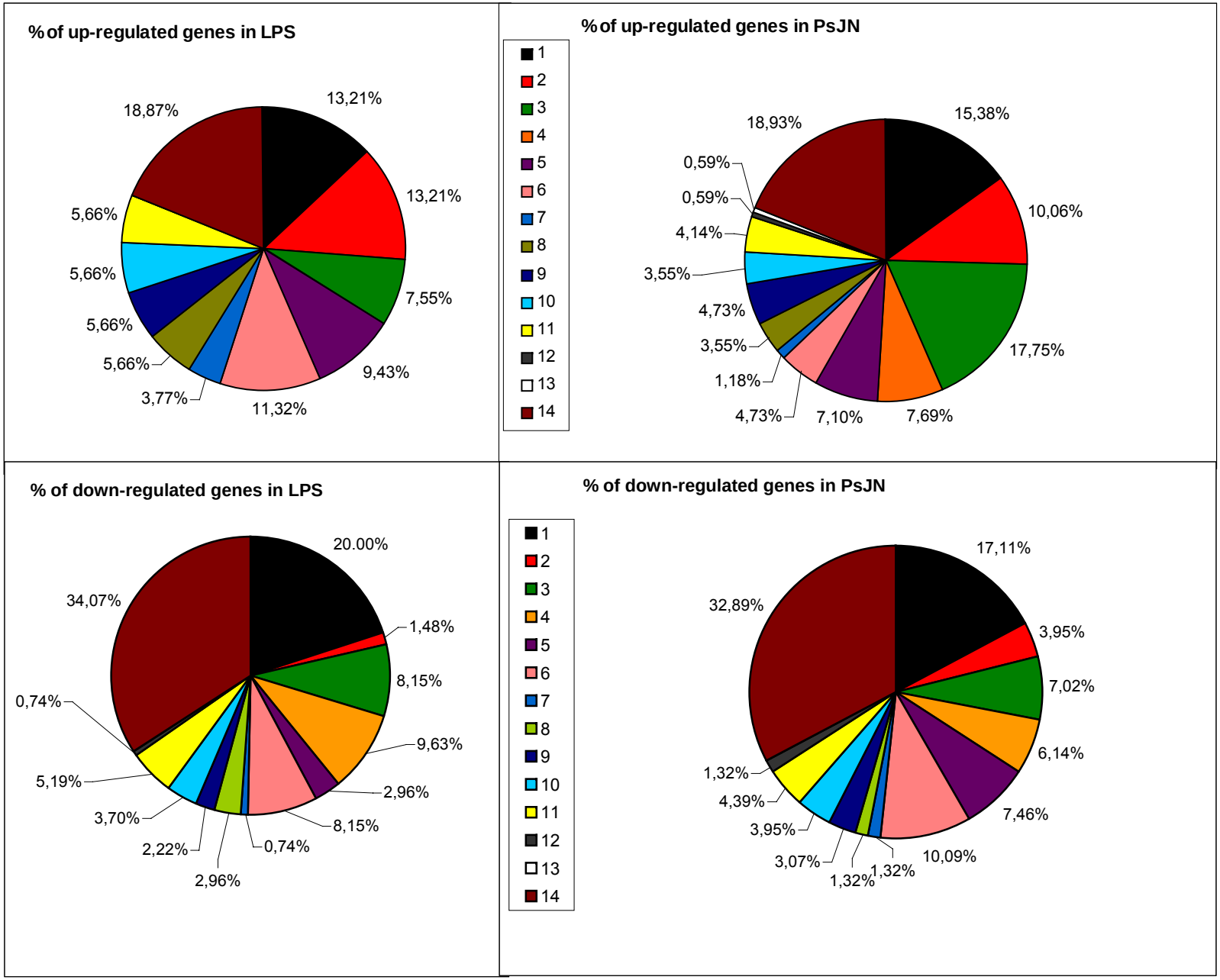

Figure 16: Percentages of up- and down-regulated genes of the different functional categories of plants inoculated with LPS and PsJN.

***1**-Metabolism*
***2**-Energy*
***3**-Protein synthesis*
***4**-Transcription*
***5**-Protein fate (folding, modification, destination)*
***6**-Protein with binding function or cofactor requirement (structural or catalytic)*
***7**-Regulation of metabolism and protein function*
***8**-Subcellular localization*
***9**-Cell rescue and defense*
***10**-Interaction with the environment*
***11**-Cellular transport, transport facilities and transport routes.*

3.4.1 Systemic resistance

According to several publications, LPS have the ability to induce systemic resistance in various plant species (Leeman *et al.*, 1995, Reitz *et al.*, 2000, Desaki *et al.*, 2006) and also to increase the speed and/or degree of induction of defense responses induced by following pathogen encounter (Newman *et al.*, 2002). Thus, LPS together with various other biological agents and synthetic compounds can form priming in plants (Conrath *et al.*, 2006).

In this study no indication priming could be detected in the transcriptome analysis. The molecular mechanisms of priming are not yet fully understood and it is apparent that this process is not of a trivial nature. It is hypothesized, however, that defense responses are not activated directly in primed plants, but are speeded up upon an attack, providing the plant with an enhanced capacity to respond to an invading pathogen (Conrath *et al.*, 2006, Frost *et al.*, 2008). Thus, defense responses are expressed only when really needed (i.e., upon pathogen attack) and only those defense responses are enabled that are triggered by a specific pathogen. It is likely that priming is not regulated at the transcriptional level, although changes in gene expression might occur below the level of detection (Verhagen *et al.*, 2004). An alternative explanation could be that priming might be regulated post-translationally (Verhagen *et al.*, 2004) .

Though endophytic bacteria can enter the host plant through the stomata of leaves, the more probable event is an infection from the soil into the roots of the host. We infiltrated the leaves using a syringe to make sure that a defined amount of the endophyte was ingested into the plants, but this direct insertion does not guarantee that the bacteria survived long enough to interact with the plant to have a priming effect. In natural conditions, the bacteria entering the plants through the rhizosphere have more time to adapt to the new environment inside the plant.

3.4.2 Transcriptomic changes in potato induced by endophytes

Other studies dealing with the transcriptomic changes in plants interacting with endophytes showed that in most cases only transient, weak or strictly localized responses were produced (Verhagen *et al.*, 2004, Wang *et al.*, 2005), which stands in contrast to the massive induction of defense responses triggered during plant–pathogen interactions. Unlike the systemic immune responses triggered upon pathogen encounter, the processes within plants activated by beneficial

microorganisms mostly were not associated with constitutive reprogramming of the transcriptome; changes of gene expression were either not detectable at all (Verhagen et al., 2004) or at low level (Wang *et al.*, 2005, Alfano *et al.*, 2007, Liu *et al.*, 2007, Van der Ent *et al.*, 2008).

Experiments of Verhagen *et al*. (2004) demonstrated that the transcript levels of several defense-related genes (e.g. *HEL*, *PDF1.2*, *PR-1*, *PR-2*, and *PR-5*) were not elevated in the leaves in response to colonization of the roots by induced systemic resistance (ISR) activating rhizobacteria. Pathogen-induced SAR and rhizobacteria-mediated ISR are inducible defense responses that are controlled by different signaling pathways (Pieterse *et al.*, 1998). Expression analysis of *Arabidopsis* during build-up of SAR revealed that this type of induced resistance goes along with considerable transcriptional reprogramming (Maleck *et al.*, 2000). This results in the accumulation of SAR gene products from 0.3-1% of the total mRNA and protein content (Lawton *et al.*, 1995).

Our findings show similar outcomes. Only very few defense-related genes were upregulated in treatments with PsJN which include a few protease and proteinase inhibitors and endochitinase. These defense-related genes were present within the categories “cell rescue and defense” and “Interaction with the environment“ and were up-regulated with an abundance of 4.7% and 3.6%, respectively. One pathogenesis-related (PR) 4 cluster was down-regulated, another one was 1.3-fold up-regulated. In rice the gene PR-1, mediated by NO and a marker for SAR, was not induced during endophytic symbiosis (Güimil *et al.*, 2005), in our study PR-1 was 1.3-fold down-regulated.

3.4.3 Transcriptomic changes in potato induced by LPS

LPS treatment in Arabidopsis generally induced stress- or defense -associated genes including glutathione S-transferases, cytochrome P450-type enzymes, and many genes encoding pathogenesis related (PR) proteins (Zeidler *et al.*, 2004, Mishina & Zeier, 2007). In our analyses glutathione S-transferase was 1.7-fold down-regulated and cytochrome P450 was not significantly induced.

During an incompatible interaction of pepper with *Xanthomonas campestris*, an increase in transcription of genes encoding tyraminehydroxycinnamoyl transferase (THT) and phenylalanine-ammonia lyase (PAL) was found in the plant (Newman *et al.*, 2001). THT was not significantly down-regulated in this study.

In contrast to the finding of Newman *et al.* (2007) where LPS activated the induction of PR-1 in rice, LPS alone was not able to induce the accumulation of the PR-1 transcript in potato. Respectively 5.7% of the genes in the categories “cell rescue and defense” and “Interaction with the environment“ were up-regulated.

In another study, LPS led to low level transcripts of defense-related genes in Arabidopsis, most of them repressed or not expressed at all (Livaja *et al.*, 2008), which is somewhat similar to our results as well. We found only few defense genes such as PR-4, which was down-regulated in treatments with LPS. Endochitinase 2 and a proteinase inhibitor were, on the other hand, up-regulated. Analogous to that, Livaja *et al.* (2008) found that only very few PR-proteins (PR-3-type chitinase and PR-4 hevein) were induced through LPS.

In tobacco and Arabidopsis, LPS induced a gene encoding superoxide dismutase, involved in the production of hydrogen peroxide (Newman *et al.*, 2007, Livaja *et al.*, 2008), which was also up-regulated in this study.

Xyloglucan endotransglycosylase was found to be induced in Arabidopsis, which is responsible for the depolymerization of the hemicelluloses processing xyloglucan in the cell wall (Micheli, 2001, Livaja *et al.*, 2008). In our study the gene encoding xyloglucan endotransglycosylase was strongly down-regulated.

These data compared to the results with PsJN infiltration implies that LPS alone cannot evoke the same power of response in the plant compared to the whole bacterium, since this probably requires other components of the bacterial cell.

3.4.4 Crosslinking the microarray with the ROS, NO and SA measurements

It is known that receptor kinase, genes for peroxisome biogenesis (Desikan *et al.*, 2000) and annexin (Moseyko *et al.*, 2002) are ROS-induced genes. None of these genes were found active in our samples.

However, some genes for ROS scavenging were present on the microarray. SOD, which was up-regulated in both treatments and ascorbate peroxidase, which was down-regulated in plants infiltrated with PsJN.

The measurements of ROS with the dye H_2DCFDA mainly involved the reactions of the plants with the different treatments to infection with *P.infestans*. It is apparent that ROS was not induced in the time-frame the microarrays were made, probably because the samples used for the microarrays were not challenged with the pathogen.

NO induces phenylalanine ammonia lyase (PAL), which was up-regulated in treatments with PsJN. This suggests that LPS was not able to induce NO in a stronger way than the control solution.

Since NO and SA interact closely, PR-1 is a marker gene for both. In accordance with the results of the SA quantification, PR-1 was down-regulated significantly in samples with PsJN, thus indicating the supression of SA.

PR-4 is a marker gene for JA and it was down-regulated both in LPS and PsJN treatments. Chitinase B (CHIB) is also induced upon JA and it was up-regulated in PsJN. Since measurement of JA was below detection limit, we have no data to compare.

It is important to point out that the effects on plants can depend on the origin of the LPS (Newman *et al.*, 2007). Bacterial symbionts have evolved a wide range of strategies to overcome or suppress plant defense response. While some of these strategies are shared with pathogenic bacteria, others are very specific for beneficial bacteria (Soto *et al.*, 2006).

Furthermore, the microarray used in this study represents only part of the potato's genome and it was originally designed to detect defense responses in potato upon challenge by pathogens; therefore it is possible that important genes for plant-endophyte interactions may not be present on this microarray.

To confirm observations made by microarray hybridizations it is useful to apply different, more sensitive methods of measuring gene expression levels. These include Northern blotting and RT-PCR as the most common tools for data verification (Maleck *et al.*, 2000, Kawasaki *et al.*, 2001).

4 Conclusions and further prospects

This study intends to provide a better understanding of the effects of endophyte PsJN and its LPS to the potato transcriptome.

We found that ROS and NO were stronger induced in plants infiltrated with the bacterium *Burkholderia phytofirmans* strain PsJN compared to treatments with LPS and the control solution PBS. SA was suppressed by PsJN and LPS, but the effect lasted longer in infiltrations with PsJN.

To investigate a beneficial effect of LPS and PsJN, the plants were infected with the oomycete *P.infestans* after infiltration. The leaves were sampled for a detached leaflet assay and quantitative real-time PCR was performed, targeting the oomycete. *P.infestans* seemed to have grown better on leaves infiltrated with LPS, thus no positive effect was proved in this assay. We suspect that the detached leaflet assay was not sufficient for this kind of investigation, since a detached leaf is not provided anymore by metabolites which are important to a successful defense.

The transcriptome analysis showed that only very few defense-related genes were up-regulated in treatments with PsJN, which include one cluster of a protease inhibitor, three clusters of proteinase inhibitors and five ESTs of endochitinase 2. One pathogenesis-related (PR)-4 cluster with 18 ESTs was down-regulated while another cluster with four ESTs was up-regulated. Similarly to that, in treatments with LPS the defense-related genes endochitinase 2 represented by five ESTs and a proteinase inhibitor with two ESTs were up-regulated, whereas the PR-4 cluster with 18 ESTs was down-regulated.

We have demonstrated that the plants responded stronger to the colonization by living endophytic bacteria, relative to response upon infiltration of LPS alone. This implies, that there are probably more components in the bacterial cell necessary for the interaction with the host plant.

Moreover, this study also showed the importance of time series, as transcriptomic changes in plants often occur transiently and to detect changes at all, catching the right moment is crucial.

Biological control of plant pathogens involves the use of natural resources, such as certain beneficial organisms like strain PsJN. Because this way of sustainable agriculture is an environmentally and consumer friendly alternative to chemical

pesticides and fertilizers, a better understanding of the molecular aspects of microbe-induced disease suppression is crucial for the development of a successful biocontrol strategy.

5 Appendix

Table 7: Data sheet of significantly up- and down-regulated genes classified under the different functional categories of LPS and PsJN treated plants.

Metabolism

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
BM111915	BM111915	Xyloglucan endotransglucosylase-hydrolase XTR3	0,00E+00		-4,6	-10,9
CK272973	CK272973	S-adenosylmethionine synthetase 2	0,00E+00			-1,1
BQ513975	BQ513975	Ribulose-phosphate 3-epimerase, cytosolic	0,00E+00	2	-1,4	-1,9
5P15		Cystathionine beta-synthase (Serinesulfhydrase) (Beta-thionase)	0,00E+00			-1,8
BG592238	BG592238	similar to UP|Q8RWN6_ARATH (Q8RWN6) Strong similarity to naringenin 3-dioxygenase, partial (51%)	7,00E-11		-1,5	-1,8
CK279392	CK279392	dTDP-glucose 4-6-dehydratase	2,00E-167		-1,5	-1,8
6B22		Amine oxidase family protein	4,00E-144			-1,7
9A24		UDP-glucose 6-dehydrogenase	0,00E+00			-1,7
BQ122080	BQ122080	Glycerophosphoryl diester phosphodiesterase family protein	1,00E-110	2		-1,7
2N6		Dehydrodolichyl diphosphate synthase	9,00E-27		-1,5	-1,6
2B9		ATHAL3A [Arabidopsis thaliana]	4,00E-172			-1,6
8K10		Vacuolar ATP synthase 16 kDa proteolipid subunit	9,00E-119	2	-1,3	-1,5
BQ115285	BQ115285	similar to UP|Q1W5D1_HEVBR (Q1W5D1) Solanesyl diphosphate synthase, partial	0,00E+00		-1,7	-1,5
BQ112920	BQ112920	Thiazole biosynthetic enzyme, chloroplast precursor	5,00E-119			-1,5
6M2		putative succinyl-CoA ligase (GDP-forming) beta-chain,mitochondrial precursor [Oryza sativa (japonicacultivar-group)]	0,00E+00		-1,3	-1,5
BM404854	BM404854	Cytosolic cysteine synthase,complete	0,00E+00			-1,5
3L19		Cytosol aminopeptidase	0,00E+00	3		-1,5
BQ115515	BQ115515	Xyloglucan endotransglycosylase LeXET2	0,00E+00		-1,3	-1,5
BQ119277		Delta-1-pyrroline-5-carboxylate dehydrogenase	0,00E+00			-1,5
9K11		Malate oxidoreductase	5,00E-167			-1,5
BQ114483	BQ114483	Dehydroascorbate reductase				-1,4

10C10		Nitrilase	8,00E-07		-1,2	-1,4
9A9		CYN (CYANASE); cyanate hydratase	5,00E-51	2	-1,3	-1,4
9I13	FL670588	ARABIDOPSIS THALIANA CARBOXYESTERASE 20	1,00E-56			-1,4
1B3	AM907411	OPR2; 12-oxophytodienoate reductase [Arabidopsis thaliana]	2,00E-103			-1,4
BG890019	BG890019	Amidohydrolase family protein	3,00E-50			-1,4
6F11		2;3-bisphosphoglycerate-independent phosphoglycerate mutase	0,00E+00			-1,4
8H16		Endoribonuclease L-PSP family protein	0,00E+00			-1,4
1K11		Trypsin-like protease [Streptomyces avermitilis MA-4680]	0,00E+00		-2,1	
BQ114297	BQ114297	Phosphoethanolamine N-methyltransferase 1 / PEAMT 1 (NMT1)	0,00E+00		-1,4	
F1172		Ribulose-1,5-bisphosphate carboxylase			-1,6	
5P20		Lactoylglutathione lyase [Arabidopsis thaliana]	2,00E-39		-2,1	
BQ511857		Fructose-1,6-bisphosphatase, putative	0,00E+00		-1,2	
3A15		Calcineurin-like phosphoesterase family protein	0,00E+00			-1,2
3A24		ATGSTU1 (GLUTATHIONE S-TRANSFERASE 19); glutathione transferase[Arabidopsis thaliana]	0,00E+00		-1,7	
9I18		ATP synthase epsilon chain, mitochondrial	9,00E-95		-1,5	
3N23		GAD (Glutamate decarboxylase 1); calmodulin binding	0,00E+00		1,4	
Rubisco		Rubisco		29	1,3	
CK265323	CK265323	porphobilinogen synthase, putative	1,00E-101		1,3	
CK271046	CK271046	cobalamin biosynthesis protein	4,00E-168			1,2
BQ112708	BQ112708	Acid phosphatase	0,00E+00			1,2
BQ113388	BQ113388	Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase	0,00E+00			1,2
9D5		Phenylalanine ammonia-lyase, putative	6,00E-152			1,3
BQ113763	BQ113763	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	5,00E-30			1,3
BQ111476	BQ111476	GDP-mannose 3;5-epimerase 1	0,00E+00			1,3
4C18		ATGSTT1; glutathione transferase	4,00E-52			1,3
10D23		Beta-fructosidase (BFRUCT3)	0,00E+00			1,3
1I8		Beta-fructosidase (BFRUCT4)	0,00E+00			1,4
BQ112798	BQ112798	Glucose-1-phosphate adenylyltransferase large subunit 3	2,00E-157			1,4
6O6		Protoporphyrinogen oxidase; chloroplast precursor	0,00E+00			1,4
BQ111350	BQ111350	ATAPY2 (APYRASE 2)	2,00E-93			1,4
9M10		Caffeoyl-CoA 3-O-methyltransferase, putative	0,00E+00			1,4

BQ116194	BQ116194	B1144G04.32 [Oryza sativa (japonica cultivar-group)]	8,00E-10		1,3	1,5
BQ506628	BQ506628	Putative Squalene monooxygenase	0,00E+00		1,5	1,7
BQ114144	BQ114144	Delta(14)-sterol reductase	6,00E-10			1,8
CK264748	CK264748	protochlorophyllide reductase B	1,00E-105		1,5	
BQ111514	BQ111514	4-aminobutyrate aminotransferase / gamma-amino-N-butyrate transaminase	0,00E+00			1,9
BQ113576	BQ113576	Sterol desaturase family protein	0,00E+00			2,0
BQ112901	BQ112901	GDSL-motif lipase/hydrolase family protein	2,00E-05			2,5
BQ112142	BQ112142	2-oxoglutarate-dependent dioxygenase,complete	2,00E-149			2,6
BQ113480	BQ113480	2-oxoglutarate-dependent dioxygenase	0,00E+00			2,7
BQ113913	BQ113913	phosphorylase family protein	1,00E-24			3,1
BQ111821	BQ111821	Flavonol-3-O-glycoside-7-O-glucosyltransferase 1	0,00E+00			3,3
CK272973	CK272973	S-adenosylmethionine synthetase 2	7,00E-102	2	-1,3	-1,4
CK269293	CK269293	coclaurine N-methyltransferase, putative	1,00E-120		1,2	1,3
CK268337	CK268337	UDP-D-APIOSE/UDP-D-XYLOSE SYNTHASE 2	1,00E-159		-1,4	-1,9
CK272870	CK272870	long-chain-fatty-acid--CoA ligase / long-chain acyl-CoA synthetase	4,00E-101			-1,3
CK265608	CK265608	ornithine decarboxylase (SPE1)	1,00E-51			1,3
CK252043	CK252043	Cysteine desulfurase [Zea mays] zu metabolism	2,00E-25		-1,4	-1,6
CK269917	CK269917	Aldehyde dehydrogenase, putative / antiquitin	1,00E-99		-1,2	
CK266756	CK266756	Peroxidase 12 (PER12) (P12) (PRXR6)	2,00E-141	2		1,2
CK268454	CK268454	12-oxophytodienoate reductase 1	2,00E-128		-1,4	-1,4
5N5	AM906549	NADH-ubiquinone oxidoreductase-related	9,00E-44		-1,4	-1,6
1I3	AM907455	NADH-ubiquinone oxidoreductase-related	6,00E-44		-1,4	-1,5
5K14	AM908665	NADH-ubiquinone oxidoreductase	1,00E-35	2	-1,2	-1,5
9G3	AM907026	NADH dehydrogenase (ubiquinone)	7,00E-68		-1,3	-1,4
6N14	AM908999	NADH dehydrogenase (ubiquinone) 18 kDa subunit	5,00E-68			-1,3

Energy

Genes.Name	NCBI	Annotation	e value KEGG	#ESTs	FCR LPS	FCR PsJN
BM408185	BM408185	Probable vacuolar ATP synthase subunitH	2E-82			-1,8
5C2	AM906489	Cytochrome C Oxidase 6B	1E-35			-1,4
4N5	AM908426	Photosystem I reaction center subunit II	1E-29			-1,3
8O23	AM906986	Photosystem II 10 kDa polypeptide	5E-48	2	-1,3	-1,3
3J21	AM908314	ATP synthase F0 subunit 1	3E-92			-1,3
7N18	AM906722	Vacuolar ATP synthase subunit E	3E-70			-1,3
9L5	AM907053	Cytochrome c1 heme protein	4E-90	2		-1,3
CK266013	CK266013	Ribulose bisphosphate carboxylase/oxygenase activase	5E-123			-1,3
14_O11-S12	BQ121702	clp-like energy-dependent protease	1,00E-100			-1,3
4D6	AM908372	ATP synthase epsilon chain, mitochondrial	2E-26		-1,4	
3D24	AM908288	Cytochrome b6-f complex iron-sulfur subunit	2E-24			1,2
7N19	AM909331	Photosystem I reaction center subunit III	2E-76		1,2	
BQ112941	BQ112941	ATP synthase gamma chain	2E-29	2		1,2
TP14H05		Chlorophyll a-b binding protein	5,00E-117			1,2
TP22G03		chlorophyll a/b-binding protein	2,00E-50		1,3	1,3
BQ113336	BQ113336	Photosystem II protein PsbX	2E-15			1,3
CK272685	CK272685	Chlorophyll a/b binding (CAB) polypeptides of CP29 polypeptide	2E-78			1,3
TP14B04		Light harvesting chlorophyll a/b-binding protein	2,00E-52		1,2	1,4
BQ113398	BQ113398	Oxygen-evolving complex related protein	9E-64			1,4
BQ114303	BQ114303	Photosystem II reaction center PSB28 protein	4E-24			1,4
BG096395	BG096395	Chlorophyll a/b-binding protein (cab-12)	2E-82		1,3	1,4
TP11H01		chlorophyll A-B binding protein	1,00E-06		1,4	1,4
BQ112210	BQ112210	Photosystem I reaction center subunit X psaK	7E-25			1,5
2N9	AM907913	Stress Enhanced Protein 2	1E-28			1,5
BQ111722	BQ111722	Chlorophyll a-b binding protein CP26	2E-62		1,2	1,5
CK275173	CK275173	Chlorophyll A-B binding protein CP26	6E-94		1,2	1,2
CK274770	CK274770	chlorophyll A-B binding protein 2, chloroplast	8,00E-128			1,2

CK272309	CK272309	chlorophyll A-B binding protein / LHCI type I (CAB)	3,00E-36			1,2

Protein synthesis

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
7N15	AM906716	40S ribosomal protein S17	6,00E-64			-1,5
BM408482	BM408482	60S ribosomal protein L17	8,00E-77		-1,2	-1,2
9E22	AM909533	60S ribosomal protein L17	4,00E-74		-1,3	-1,2
5O22	AM908765	60S ribosomal protein L37-2	4,00E-41	2	-1,3	-1,5
3M11	AM908173	40S ribosomal protein S17	8,00E-64			-1,5
5M10	AM906635	40S ribosomal protein S8	6,00E-80			-1,4
BQ519040	BQ519040	60S ribosomal protein L14-1	6,00E-55	2	-1,2	-1,4
5I20	AM908733	60S ribosomal protein L18-2	6,00E-70			-1,4
BE340312	BE340312	60S ribosomal protein L15	2,00E-89			-1,3
5N20	AM908752	Eukaryotic translation initiation factor 5A-4	6,00E-88	2		-1,3
BG593491	BG593491	40S ribosomal protein S19-3	1,00E-55			-1,3
5H2	AM906511	60S ribosomal protein L8	1,00E-129			-1,3
6G5	AM908811	60S ribosomal protein L12	5,00E-81	3	-1,3	-1,3
7C22	AM909283	Chloroplast 50S ribosomal protein L2 A	1,00E-104			-1,2
18S		18S ribosomal protein		45	-1,4	-1,2
BI177008	BI177008	40S ribosomal protein S24	7,00E-58		-1,2	-1,2
BQ113869	BQ113869	Chloroplast 50S ribosomal protein	1,00E-72		-1,3	
6M13	AM908996	60S acidic ribosomal protein P2	1,00E-22	2	-1,3	
1L13	AM907640	40S ribosomal protein S14-2	3,00E-57	3	-1,3	
10N21	AM907392	60S ribosomal protein L6 (YL16-like)	2,00E-37		-1,3	
1K15	AM907643	Plastid-specific 30S ribosomal protein 1; chloroplast precursor	3,00E-53	2	1,3	
6G8	AM908888	60S ribosomal protein L26-1	1,00E-42			1,2
7A16	AM906657	60S ribosomal protein L11-1 (L16A)	1,00E-88	2		1,2
2P14	AM908010	60S ribosomal protein L14-1	7,00E-52			1,2
BQ112343	BQ112343	50S ribosomal protein L19-1; chloroplast precursor	2,00E-37		1,3	1,3

9C8	AM907092	60S ribosomal protein L18	4,00E-58		2,0	
5E3	AM906501	50S ribosomal protein L23	2,00E-06			1,3
9P11	AM907168	30S ribosomal protein S17; chloroplast precursor	3,00E-30			1,3
6C11	AM908873	40S ribosomal protein S15a-1	2,00E-69	6		1,3
4D23	AM908543	50S ribosomal protein L27; chloroplast precursor	4,00E-66		1,4	1,3
1I15	AM907631	60S ribosomal protein L34	7,00E-46			1,3
1D5	AM907427	60S ribosomal protein L7-4	6,00E-56			1,3
7D10	AM909201	Elongation factor 1-alpha (EF-1-alpha)	8,00E-67			1,3
7G16	AM906687	60S ribosomal protein L37a	3,00E-47			1,4
6I8	AM908897	40S ribosomal protein S18	5,00E-67			1,4
8N23	AM906976	40S ribosomal protein S10-3	4,00E-48	2		1,4
1L23	AM907733	Ribosome biogenesis regulatory protein (RRS1) family protein	8,00E-54			1,4
8C15	AM909364	40S ribosomal protein S27-2	1,00E-38			1,4
5N6	AM906551	60S ribosomal protein L28-1	4,00E-56			1,4
BM406913	BM406913	40S ribosomal protein S27-2	1,00E-43			1,4
BQ518909	BQ518909	40S ribosomal protein S12	1,00E-06			1,5
4A11	FL670509	40S ribosomal protein S18	2,00E-68	2		1,5
2C15	AM907946	40S ribosomal protein S16	3,00E-70	2		1,5
7P12	AM909268	60S ribosomal protein L22-2	2,00E-31	2		1,5
6H19	AM909049	60S ribosomal protein L32-1	9,00E-49			1,5
3B17	FL670497	40S ribosomal protein S12	1,00E-40			1,5
BQ113562	BQ113562	60S ribosomal protein L30	2,00E-50	2		1,6
5F22	AM908718	50S ribosomal protein L31	2,00E-17			1,6
BQ506881	BQ506881	60S ribosomal protein L6-2	4,00E-09			1,6
BG888686	BG888686	40S ribosomal protein S14-2	4,00E-59	2		1,7
3L16	AM908246	60S ribosomal protein L6 (YL16-like)	4,00E-68			1,9
CK262310	CK262310	60S ribosomal protein L3 (RPL3A) [KO:K02925]	6,00E-145			1,5

Transcription

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
BQ121555	BQ121555	Translation initiation factor [Arabidopsis thaliana]	3E-51		-1,3	-1,8
BQ512461	BQ512461	WRKY17; transcription factor	2E-31			-1,7
9B11	AM907087	Myb family transcription factor	3E-21			-1,6
BQ518765	BQ518765	MYB111; DNA binding / transcription factor	2E-46		-1,5	-1,5
BG592029	BG592029	Two-component responsive regulator / response regulator 15 (ARR15)	7E-54		-1,5	-1,5
BQ113469	BQ113469	WRKY transcription factor 33	4E-26			-1,4
CK261290	CK261290	AP2 domain-containing transcription factor RAP2.4	8,00E-44		-1,5	-1,4
9O7	AM907159	Eukaryotic translation initiation factor 1	1E-52		-1,3	-1,3
CK262175	CK262175	Elongation factor 1-alpha (EF-1-alpha)	1,00E-122			-1,3
5G24	AM908729	BolA-like family protein	4E-28			-1,3
10H15	AM907286	Ethylene-responsive transcriptional coactivator	1E-58			-1,3
6A20	FL670537	Small nuclear ribonucleoprotein G, putative	3E-34		-1,4	-1,2
BQ119839	BQ119839	PHD finger family protein / bromo-adjacent homology (BAH) domain-containing protein	1,00E-69			-1,2
7M9	AM909251	No apical meristem (NAM) protein family; ANAC018	9E-66	2	-1,4	-1,2
9P4	AM907074	Cwf15 / Cwc15 cell cycle control family protein	1E-36		-1,3	
5G2	AM906510	Rubber elongation factor (REF) family protein	7E-32		-1,6	
BQ111649	BQ111649	NAC domain containing protein 83; transcription factor; ANAC018	9E-51		-1,3	
1I24	AM907724	zinc finger (C2H2 type) family protein	2E-39		-1,4	
10M19	AM907387	NAC domain containing protein 83); transcription factor	7E-58		-1,4	
9C15	FL670584	AG-motif binding protein-2 [Nicotiana tabacum]				1,2
CK263184	CK263184	Transcription initiation factor IIB-2	1E-109		1,2	
9C1	AM907000	Translational initiation factor eIF1	2E-51	2		1,2
BQ113492	BQ113492	poly (A) polymerase family protein	7E-33			1,2
BM404685	BM404685	Dehydration-responsive protein-related	2E-126			1,3
ElonFac	34	Elongation factor				1,3
CK265999	CK265999	BEL1-like homeobox 4 protein (BLH4) transcription factor	3,00E-61			1,3
8J6	AM906788	DNA binding / transcription factor [Arabidopsis thaliana]	9E-25			1,3

8C11	AM906839	Putative transcription factor	5E-48		1,3
CK261282	CK261282	Eukaryotic translation initiation factor 3 subunit 6 (eIF-3 p48)	0		1,4
1O12	AM907581	eukaryotic cap-binding protein	6E-79		1,5
8E18	AM909377	Eukaryotic translation initiation factor 4E type 3	6E-59		1,5
5I17	AM908662	myb family transcription factor	1E-37		1,6
3A11	AM908107	Short vegetative phase protein (SVP) ; transcription factor	5E-19		1,6

Protein fate (folding, modification, destination)

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
8O6	AM906821	Peptide-methionine-(S)-S-oxide reductase	1,00E-46		-1,5	-1,9
2A9	AM907842	Peptidyl-prolyl cis-trans isomerase	7,00E-52		-1,3	-1,8
3K2	AM908072	Ubiquitin-like protein	7,00E-35		-1,4	-1,8
CK256999	CK256999	CBL-interacting protein kinase 7	3,00E-98		-1,3	-1,5
3H20	AM908303	OTU-like cysteine protease family protein	1,00E-50		2,1	
cyclo	38	cyclophilin				-1,4
2F10	AM907869	Ubiquitin-like protein (SMT3)	4,00E-23			-1,3
3G16	AM908223	Band 7 family protein, hypersensitive-induced response protein	2,00E-88			-1,3
CK266774	CK266774	ATP-dependent Clp protease ATP-binding subunit	5,00E-157			-1,3
10B8	AM907173	weak similar to ubiquitin-conjugating enzyme E2-17 kDa 9	5,00E-06			-1,3
3K14	AM908242	Immunophilin / FKBP-type peptidyl-prolyl cis-trans isomerase	1,00E-81			-1,3
4O16	AM908521	Ubiquitin family protein contains INTERPRO:IPR000626 ubiquitin domain, partial (87%)	9,00E-44			-1,3
CK265445	CK265445	SNF7.1 [Arabidopsis thaliana]	7,00E-60			-1,3
8J10	AM906871	Peptidyl-prolyl cis-trans isomerase / cyclophilin (CYP1) / rotamase	4,00E-72	2		-1,2
3C23	AM908285	Protein-methionine-S-oxide reductase [Arabidopsis thaliana]	8,00E-46			-1,2
4G21	AM908560	DNAJ heat shock N-terminal domain-containing protein	6,00E-55			-1,2
2I13	AM907976	Ubiquitin conjugating enzyme/ ubiquitin-like activating enzyme	2,00E-47			-1,2
4L20	AM908582	Histone H3 [Oryza sativa (japonica cultivar-group)]	8,00E-65			-1,2
TP06G06		Heat shock protein 70, putative	5,00E-70		2,5	
CK264586	CK264586	Heat shock protein 80 [Solanum tuberosum]	0,00E+00		1,3	

BM111850	BM111850	Ubiquitin	7,00E-134			1,2
BI434541	BI434541	Ubiquitin-conjugating enzymed 2	2,00E-83			1,3
1C10	AM907512	Ubiquitin extension protein 6 (UBQ6)	8,00E-44	2		1,4
7E2	AM909127	Ubiquitin-conjugating enzyme E2 7	3,00E-72			1,4
F736	EC907615	polyubiquitin 4 - wild oat				1,4
4L7	AM906445	DSBA oxidoreductase family protein	1,00E-77		1,6	1,4
2H6	AM907796	Prefoldin, putative	1,00E-43			1,4
10P4	AM906298	Ubiquitin-conjugating enzyme 14 (UBC14)	3,00E-72			1,5
BG890352	BG890352	Heat shock factor (HSF)-type; DNA-binding	2,00E-47			1,5
F277	EC907520	Heat shock protein hsp70	2,00E-89			1,6
CK265363	CK265363	leucine-rich repeat family protein	2,00E-95			1,8
BQ114103	BQ114103	Signal peptidase complex subunit 2	3,00E-68		1,3	1,8

Protein with binding function or cofactor requirement (structural or catalytic)

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
9I19	AM909549	Heavy-metal-associated domain-containing protein	4,00E-08			-2,5
2N4	AM907826	Nonspecific lipid transfer protein 1 (LTP1)	2,00E-16		-2,1	-2,2
9M12	AM907157	Curculin-like (mannose-binding) lectin family	9,00E-67			-2,1
9N4	AM907063	Clathrin adaptor complex small chain family protein	2,00E-63		-1,6	-2,1
CK265008	CK265008	protein kinase family protein	1,00E-113			-2,0
CK267073	CK267073	putative calreticulin	3,00E-128		-1,5	-1,9
7M23	AM909338	Germin-like protein, putative	9,00E-44			-1,7
CK264561	CK264561	mitochondrial substrate carrier family protein	3,00E-53		-1,2	-1,6
5O19	AM908759	Tobamovirus multiplication protein 3 (TOM3)	3,00E-30		-1,4	-1,6
1C24	AM907692	Zinc finger (AN1-like) family protein	2,00E-47			-1,6
BQ505884	BQ505884	Phosphoenolpyruvate carboxylase kinase	9,00E-45			-1,6
7E20	AM909289	DNA binding [Arabidopsis thaliana]	5,00E-43			-1,5
10H1	AM906249	FDH (FORMATE DEHYDROGENASE); oxidoreductase	6,00E-24			-1,5
9E8	AM907104	Lipid transfer protein-related	1,00E-41			-1,5

9C6	AM907009	nucleic acid binding [Arabidopsis thaliana]	9,00E-20			-1,5
8E19	AM906930	vacuolar ATP synthase 16 kDa proteolipid subunit 3	2,00E-55			-1,4
BQ120975	BQ120975	ABC transporter family protein	7,00E-59			-1,3
2D24	AM906325	Calmodulin-7	7,00E-66			-1,3
6A8	AM908857	Small nuclear ribonucleoprotein E, putative	2,00E-39			-1,3
CK274146	CK274146	WD-40 repeat family protein [Arabidopsis thaliana]	7,00E-137			-1,3
CK249601	CK249601	Leucine-rich repeat family protein / extensin family protein	4,00E-53			-1,2
BM112479	BM112479	Calmodulin-7	1,00E-79			-1,2
BI434707	BI434707	Cytochrome P450 71A4 (CYPLXXIA4)	7,00E-62			-1,2
10G9	AM907202	CAX-interacting protein 1 (CAXIP1)	3,00E-44	2	-1,4	
13_E22-S11		14-3-3 protein	2,00E-129		1,2	
1J16	AM907634	Remorin family protein	3,00E-31		-1,4	
7A13	AM906652	calcium ion binding [Arabidopsis thaliana]	2,00E-23		2,5	
BQ115186	BQ115186	ARF GAP-like zinc finger-containing protein ZIGA3	1,00E-36		1,3	
CK264004	CK264004	ARF GAP-like zinc finger-containing protein ZIGA3 [Arabidopsis thaliana]	2,00E-48		1,3	
9A1	AM906989	kinase/ protein binding [Arabidopsis thaliana]	4,00E-15	?	-12,4	
6H8	AM908889	DNA binding / zinc ion binding [Arabidopsis thaliana]	2,00E-15			1,3
6F23	AM909045	RNA recognition motif (RRM)-containing protein	9,00E-36			1,3
CK264765	CK264765	leucine rich repeat protein (LRP), putative	1,00E-98			1,3
7B3	AM909109	Quinone reductase family protein	3,00E-72	2		1,3
1J5	AM907459	RNA binding / nucleic acid binding [Arabidopsis thaliana]	2,00E-56	2		1,3
6J19	AM909061	Calmodulin binding [Arabidopsis thaliana]	2,00E-41			1,4
CK246053	CK246053	GTP-binding family protein [Arabidopsis thaliana]	5,00E-65			1,4
BQ117890	BQ117890	Arabidopsis thaliana multidrug resistance-associated protein 9	2,00E-90		1,5	1,4
8K7	AM906875	nucleic acid binding [Arabidopsis thaliana]	2,00E-65		1,4	1,4
14_M17-S12	BQ509002	nucleic acid binding [Arabidopsis thaliana]	2,00E-29			1,6
9G18	AM909469	nucleic acid binding [Arabidopsis thaliana]	6,00E-25	2		1,7

Regulation of metabolism and protein function

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
6A3	AM908775	enzyme inhibitor/ pectinesterase/ pectinesterase inhibitor [Arabidopsis thaliana]	7,00E-06	2	-2,1	
CK262249	CK262249	Shaggy-related protein kinase alpha	2,00E-93			-1,4
8B15	AM909355	BolA-like protein	5,00E-15			-1,4
BQ113091	BQ113091	Cysteine protease inhibitor 1 precursor	8,00E-95			-1,2
BQ113896	BQ113896	Aspartic protease inhibitor 10 precursor (Wound-induced aspartate proteinase CDI inhibitor)	1,00E-71	2	1,9	2,4
BQ114028	BQ114028	Proteinase inhibitor I	7,00E-14		1,9	2,6

Subcellular localization

Genes.Name	NCBI	Annotation	e value	# ESTs	FCR LPS	FCR PsJN
10M22	AM907393	CP12-1 [Arabidopsis thaliana]	8,00E-06			-1,4
6L4	AM908828	CP12-1 [Arabidopsis thaliana]	2,00E-19	2		-1,3
4C13	AM908449	putative involvement in cytochrome oxidase assembly	4,00E-10		-2,2	
2B16	AM907938	CER1 protein, putative	3,00E-40		-1,2	
7F10	AM909212	Wound-responsive protein-related	2,00E-09		-1,2	1,2
tub_beta		beta tubulin		36	1,3	1,2
tub_alpha		alpha tubulin		42		1,5
CK261488	CK261488	tubulin alpha-6 chain (TUA6)	7,00E-146	2		1,3
1M10	AM907567	Translationally controlled tumor protein [Arabidopsisthaliana]	5,00E-67	7	1,3	1,6
BQ513205	BQ513205	Pentatricopeptide (PPR) repeat/CBS domain-containing protein	2,00E-41		1,7	1,9
CK266794	CK266794	chloroplast inner envelope membrane protein, methyltransferase	7,00E-77		-1,3	-1,6

Cell rescue and defense

Genes.Name	NCBI	Annotation	e value KEGG	#ESTs	FCR LPS	FCR PsJN
F225	EC907549	Leucine-rich repeat resistance protein-like protein	2,00E-05			-4,3
8N22	AM906975	Mitochondrion-localized small heat shock protein 23.6	1E-26			-1,8

6A6	AM908781	universal stress protein (USP) family protein	1E-54			-1,5
2G8	AM907876	Glutathione dehydrogenase (ascorbate) [Arabidopsis thaliana]	3E-72			-1,4
1H19	AM907704	Late embryogenesis abundant 3 family protein / LEA3 family protein	6E-11	2	-1,6	
2M5	AM907827	Peroxiredoxin type 2, putative	1E-72	2		-1,3
2O4	AM907836	APX1; L-ascorbate peroxidase [Arabidopsis thaliana]	1E-58			-1,2
9K3	AM907048	SEN1 (dark inducible 1)	3E-35	2		-1,2
2G6	AM907795	COR413-PM2 (cold regulated 413 plasma membrane 2)	8E-24		-1,2	
TP23E07		Thioredoxin-dependent peroxidase 1; antioxidant	2,00E-72		-1,3	
9L17	AM909611	Cu2+/Zn2+ superoxide dismutase SOD1	6E-68	5	1,3	1,1
2J4	AM907804	Superoxide dismutase [Cu-Zn], chloroplast precursor	6E-69		1,3	1,3
BQ113536	BQ113536	2-cys peroxiredoxin-related protein	1E-79	2		1,3
2L9	AM907901	Thioredoxin family protein	2E-09			1,4
9B2	AM906992	universal stress protein (USP) family protein	4E-50	2		1,5
4C24	AM908544	ATTRX1;thioredoxin H-type 1	2E-44			1,7
BQ111471	BQ111471	Senescence/dehydration-associated protein-related (ERD7)	2E-32			1,8
BQ113207	BQ113207	Plant defensin-fusion protein, putative (PDF2.3)	4E-22		1,5	2,0

Interaction with the Environment

Genes.Name	NCBI	Annotation	e value	#ESTs	FCR LPS	FCR PsJN
TP04B03		ATEP3 (Arabidopsis thaliana chitinase class IV)	3,00E-20			-2,0
4N3	AM908422	Pathogenesis related protein	6E-61	3		-1,9
5O20	AM908761	Gibberellin-regulated protein 1 (GASA1)	1E-17			-1,9
7A19	AM909269	PR4 (Pathogenesis-related 4)	3E-41	18	-1,8	-1,6
4H8	AM906425	YSL2 (YELLOW STRIPE LIKE 2); oligopeptide transporter [Arabidopsisthaliana]	1E-65			-1,5
3M3	AM908084	ATPRB1 pathogenesis-related protein , putative	2E-45		-1,5	-1,5
6O5	AM908851	Enhanced disease susceptibility protein / salicylic acid induction deficient protein	3E-26			-1,5
9M13	AM909489	ATPRB1 A. thaliana basic pathogenesis-related protein 1	5E-37	14		-1,3
3G18	AM908227	Ethylene-responsive transcriptional coactivator	4E-41			-1,2
3G20	AM908302	SAG21 (Senescence-associated gene 21)	7E-14		-1,5	

2L21	AM906367	Osmotin-like protein (OSM34)	6E-62		-1,5	
5P5	AM906559	ATP-dependent Clp protease proteolytic subunit	4E-49		-1,4	
6A7	AM908855	PR4 (Pathogenesis-related 4)	5E-10	4		1,3
1D12	FL670480	ATHCHIB basic chitinase	3E-91			1,4
1M20	AM907737	Auxin-induced protein 22D (Indole-3-acetic acid-induced protein ARG13)	9E-30	2	1,4	
4N22	AM908597	Endochitinase 2	9E-88	5	1,5	1,4
BQ113673	BQ113673	Proteinase inhibitor type-2TR8	3E-58			1,4
9N3	AM907061	Proteinase inhibitor I20; Pin2	3E-56	2	1,4	1,5
8J1	AM906779	Proteinase inhibitor I20; Pin2	1E-54			1,6

Cellular transport, transport facilities and transport routes

Genes.Name	NCBI	Annotation	e value	#ESTs	FCR LPS	FCR PsJN
2F3	AM907780	Mitochondrial carnitine/acylcarnitine carrier-like protein	2,00E-09		16,9	
BG594517	BG594517	Protein transport protein SEC61 gamma subunit	4E-21			-1,7
10P6	AM906301	ATB5-A (Cytochrome b5 A)	8E-60		-1,6	-1,6
3L9	AM908160	SNF7 family protein	4E-62	2		-1,6
9C18	AM909450	ATB5-A (Cytochrome b5 A)	2E-49	2	-1,3	-1,5
9L13	AM909482	YKT61 (similar to yeast SNARE YKT6 1)	2E-97			-1,5
BQ113030	BQ113030	Malonyltransferase MaT1	7E-31			-1,5
5F7	AM906584	Probable aquaporin SIP2-1	1E-76			-1,4
2F24	AM906337	Thioredoxin family protein	7E-33			-1,3
4F5	AM908380	Cytochrome c, putative	1E-54		-1,2	-1,3
10O15	AM907325	sec61beta family protein	1E-17	5		-1,3
BQ113430	BQ113430	Ferric reductase-like transmembrane component family	6E-61		-1,3	
BQ116248	BQ116248	FRO1; ferric-chelate reductase	4E-87		-1,3	
6O3	AM908847	mitochondrial ATP synthase g subunit family protein	8E-54		-1,3	
CK272962	CK272962	porin, putative	6E-110		-1,3	
5C21	AM908704	Oxidoreductase	7E-49		1,3	
BG590565	BG590565	Amino acid transporter family	4E-48			1,2

1L9	AM907555	Acyl-CoA binding protein / ACBP	5E-25			1,3
BQ111363	BQ111363	SEC14 cytosolic factor, putative / phosphoglyceride transfer protein	6E-17			1,3
BQ114881	BQ114881	Transferase family protein	2,00E-42	2		1,3
1G12	AM907537	Glutaredoxin, putative	5E-41	2		1,3
BQ111690	BQ111690	Phosphate/phosphoenolpyruvate translocator protein like	6E-24			1,6
BQ112265	BQ112265	Plant lipid transfer protein and hydrophobic protein	6E-40		1,9	2,5

Cellular communication/signal transduction mechanism

Genes.Name	NCBI	Annotation	e value	#ESTs	FCR LPS	FCR PsJN
7P18	AM906732	CPK16; ATP binding / calcium ion binding / calcium- andcalmodulin-dependent protein kinase	1,00E-27			-2,3
9N2	AM907059	COP9 (Constitutive photomorphogenic 9)	1,00E-77	2	-1,5	-2,1
14_M18-S12		WNK1 ser/thr protein kinase regulates ABA responses	3,00E-30			-1,4
6G22	AM909054	RALFL33 (RALF-like 33)	3,00E-18	2		1,3

Cell fate or subcellular location

Genes.Name	NCBI	Annotation	e value	#ESTs	FCR LPS	FCR PsJN
BQ116319	BQ116319	Expansin-A15 precursor (AtEXPA15)	3E-59			1,4

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Zusammenfassung

Viele Studien haben sich bereits mit den Wechselwirkungen nützlicher Mikroorganismen und ihrer Wirtspflanzen beschäftigt. Endophytische Bakterien leben in der Pflanze und zeigen per Definition keine phytopathogenen Eigenschaften. Es ist sehr wahrscheinlich, dass alle Pflanzen von zahlreichen Endophyten besiedelt sind. Einigen Endophyten wurden bereits wachstums- und gesundheitsfördernde Wirkungen auf ihre Wirtspflanzen nachgewiesen. Da endophytische Bakterien und Phytopathogene ähnliche ökologische Nischen besetzen, besteht die Möglichkeit, nützliche Bakterien zur natürlichen Schädlingsbekämpfung einzusetzen.

Burkholderia phytofirmans (Stamm PsJN) ist solch ein nützlicher Endophyt und für seine wachstums- und gesundheitsfördernden Eigenschaften in *Solanum tuberosum* und anderen Pflanzen bekannt. Die Infektion mit PsJN kann zu einem Zustand führen, der ähnlich einer Immunisierung ist. Dieses sogenannte ‚Priming' wird bei Pflanzen als Fähigkeit definiert, schneller und/ oder stärker auf ein Pathogen zu reagieren.

Bakterielle Lipopolysaccharide (LPS) spielen in vielen Pflanzen- Mikroben Interaktionen eine wichtige Rolle. LPS tragen zur hohen Impermeabilität der bakteriellen Außenmembran bei und schützen den Mikroorganismus somit vor antibiotischen Substanzen. Sie werden zu einer bestimmten bakteriellen Substanzklasse, den sogenannten pathogen-assoziierten, molekularen Strukturen (PAMPs) gezählt. Diese PAMPs sind u.a. dafür bekannt, Priming in Pflanzen auszulösen.

Die Kartoffelfäule, hervorgerufen durch den Oomyzeten *Phytophthora infestans*, ist bis heute ein sehr hartnäckiger Schädling, welcher der landwirtschaftlichen Industrie jährlich Schäden in Milliardenhöhe beschert.

Ziel dieser Arbeit war es, die Effekte von PsJN und den daraus extrahierten Lipopolysacchariden (LPS) auf die Kartoffelsorte MF-II zu studieren. Das Hauptaugenmerk dieser Studie lag darauf, die Unterschiede in der Gentranskription nach Behandlung mit jeweils LPS oder Bakterienzellen zu untersuchen. Die Ergebnisse dieser Studie zeigten, dass bei Behandlungen mit PsJN mehr Gene aktiviert bzw. induziert wurden als bei Pflanzen, die nur mit LPS infiltriert wurden. Bei beiden Behandlungen wurden jedoch nur wenige, für die Abwehr spezifische, Gene hochreguliert.

Weiters wurden nach den verschiedenen Behandlungen und nach der Infektion mit *Phytophthora infestan*s die Auswirkungen auf die Signalmoleküle Salicylsäure (SA), Stickstoffmonoxid (NO) und reaktive Sauerstoffspezies (ROS) der Kartoffel untersucht. Auch hier zeigte sich eine stärkere Aktivität von ROS und NO in Pflanzen, die vorher mit PsJN behandelt wurden. Die Messung der Salicylsäure deutete darauf hin, daß PsJN und LPS die Bildung von SA hemmen, was möglicherweise den Endophyten vor dem Immunsystem des Wirtes schützen könnte. Dieser Effekt war am längsten in mit PsJN inokuliert Pflanzen zu beobachten; nach der Infektion mit *P.infestans* bestand dieser Effekt nicht mehr.

Um den Grad der Infektion der Blätter mit *P. infestan*s zu quantifizieren, wurde die Anzahl der Oomyzeten-DNA mittels quantititiver real-time PCR ermittelt. Dies wurde auch durchgeführt, um zu sehen, ob die Pflanzen ‚priming' durch LPS bzw. PsJN erfahren haben. Interessanterweise schien *P. infestan*s sogar am besten auf Pflanzen zu wachsen, die mit LPS infiltriert wurden. Da die Blätter für diesen Assay nach Inokulierung bzw. Infiltrierung abgenommen und dann mit *Phytophthora infestans* infiziert wurden, ist es wahrscheinlich, daß diese Blätter nicht mehr mit den Metaboliten versorgt wurden, die für eine erfolgreiche Abwehr notwendig wären.

Zusammenfassend konnten wir in dieser Studie zeigen, dass die Pflanzen stärker auf die Inokulierung durch lebenden Bakterien reagierten, als auf die Behandlung mit LPS. Es sind wahrscheinlich noch eine Reihe anderer Komponenten in der bakteriellen Zelle für eine erfolgreiche Pflanzen-Mikroben-Interaktion notwendig.

Das Einbringen von nützlichen Endophyten zur Wachstumssteigerung und Pathogenabwehr könnte ein wichtiger Bestandteil einer nachhaltigen Landwirtschaft werden. Da diese Art von Pflanzenschutz eine umweltfreundlichere und somit auch konsumentenfreundliche Alternative zu chemischen Pestiziden und Düngern darstellt, ist es wichtig, weiterhin an den molekularen Mechanismen von Pflanzen-Mikroben-Interaktionen zu forschen.

Curriculum vitae

The author of the present thesis, Valerie Hubalek, was born in Vienna, Austria, on the 19th of June 1981. In 1999, she began the study of Biology at the University of Vienna and specialized on microbial ecology.

From 2008 until 2009, she worked on her diploma thesis under the supervision of Ao. Univ.-Prof. Dr. Franz Hadacek. The scientific work was carried out under the supervision of Priv.-Doz. DI Dr. Angela Sessitsch at the Austrian Research Centers GmbH, Department of Bioresources. The results of her research work are presented in this Masters thesis.