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Interaction between potato and the endophyte *Burkholderia phytofirmans*

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Keywords

Endophyte, potato, N-acylhomoserine lactones, plant growth promotion, plant gene expression

Introduction

Bacterial endophytes have been defined as „bacteria, which for all or part of their life cycle invade tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease“ (WILSON 1995). Endophytes colonize a similar ecological niche as plant pathogens and may gain entry into plants by a number of mechanisms (reviewed by STURZ et al. 2000). Strain PsJN forms endophytic and epiphytic populations when co-cultured with potato (FROMMEL et al. 1991), tomato (PILLAY and NOWAK 1997), or grapevines (COMPANT et al. 2005). The bacterium not only stimulates plant growth (AIT BARKA et al. 2000, BENSALIM et al. 1998, FROMMEL et al. 1991, LIU et al. 1995, PILLAY and NOWAK 1997), it also induces developmental changes (FROMMEL et al. 1991), leading to better water management (LAZAROVITS and NOWAK 1997) and enhanced resistance to a low level of potato pathogens (RICHARDS 1997, STEWART 1997). Despite the fact that strain PsJN promotes plant growth of many different plant species, it has been repeatedly observed that plant performance greatly varies between different cultivars (CONN et al. 1997, PILLAY and NOWAK 1997, BENSALIM et al. 1998).

N-acyl-L-homoserine lactones (AHLs) are produced by a range of Gram-negative bacteria and are used to regulate expression of various genes in a cell-density dependent manner. This phenomenon is known as quorum sensing (QS) and is frequently found in plant-associated bacteria including pathogens. The structures of AHLs vary in the chain length of their acyl chain and chain lengths of 4-16 carbon atoms (EBERL 1999). The principal aim of this study was to study the molecular interaction between the plant and microbial genotype as well as to study the effect of AHLs.

Two, closely related, AHL-producing *B. phytofirmans* strains were selected and inoculated on two potato varieties, which respond differently to strain PsJN. Furthermore, a derivative of strain PsJN was included, which contains a lactonase gene and which showed reduced AHL production. In addition to determining the effect of bacterial inoculation on plant growth, gene expression was analyzed by using an EST-based microarray targeting more than 4000 potato sequences. Results clearly showed a different response of the two varieties to the microbial inoculation treatments, particularly to a PsJN derivative containing a lactonase gene indicating that the composition and abundance of AHLs modulate cultivar specific plant responses.

Material and Method

Analysis of N-acyl-L-homoserine lactones by UHPLC and FT/ICR-MS

For the analysis of AHLs, the strains were inoculated in 50 ml of Nutrient Broth (Sigma-Aldrich, Steinheim, Germany) and 50 ml of M9 medium containing tryptophane and grown at 30°C and 175 rpm overnight. Bacteria were harvested at 5000 g and 4°C for 5 minutes and the supernatant was used for AHL extraction. Ultra-high high pressure liquid chromatography (UHPLC) and Fourier transform ioncyclotron resonance mas spectroscopy (FT/ICR-MS) was applied for the analysis of AHLs as described previously (FEKETE et al. 2007).

Quenching of AHL-mediated signalling in B. phytofirmans PsJN

To determine the role of AHL-mediated signalling in B. phytofirmans PsJN we employed a quorum quenching approach as described by WOPPERER et al. (2006).

Plant experiments

Two potato varieties were selected for inoculation treatments. *Solanum tuberosum* cv Bionta is an Austrian cultivar highly resistant to potato virus Y, *Phytophtora infestans* and to *Globodera rostochiensis* race 1, but susceptible to wart. *S. tuberosum* cv Russet Burbank is a mutant of Burbank (USA variety) and highly susceptible to potato viruses, *Erwinia* and *Phytophthora infestans* but resistant to *Streptomyces scabies* and *Synchytrium endobioticum* (http://www.europotato.org/menu.php).

For the in vitro inoculation of explants, bacterial inoculum was prepared according to CONN et al. (1997). In brief,

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from an overnight culture bacterial cells were collected and resuspended in PBS buffer to obtain 10^8 cfu/ml. Nodal explants were immersed in the bacterial solution for 15 sec and then placed in the test tubes containing MS medium (MURASHIGE and SKOOG 1962) supplemented with 0.8% agar and 1% saccharose. Control plantlets were inoculated with PBS buffer. For the gene expression experiments plants were collected 18 days after inoculation. A second experiment was performed in the same manner to measure plant growth parameters after two months.

RNA isolation, cDNA microarray construction and hybridization protocols are deposit at the NCBI GEO server under the following ArrayDesign name: RLP array Version I; ArrayExpress accession: GPL7326 and GSM322635 (http://www.ncbi.nlm.nih.gov/geo/).

Results and Discussion

Characterization of bacterial strains and effects on plant growth

AHL analysis revealed that the quorum sensing signals produced by both strains include O-C14-HSL, OH-C14-HSL and OH-C12-HSL, whereas strain PsJN produced in addition OH-C8-HSL. Strain PsJN-*aiiA* carrying a lactonase gene shows reduced production of OH-C14-HSL, however, the production of other AHL compounds was not affected.

Both *B. phytofirmans* wildtype strains increased plant growth in Russet Burbank, whereas no significant effects were determined for the variety Bionta. Strains PsJN and RG6-12 promoted the shoot length of Russet Burbank (*Figure 1*), but shoot weight was only significantly increased by strain RG6-12. Russet Burbank inoculated with PsJN showed reduced root length. Strain PsJN-*aiiA* showed no effect on shoot production, however, caused inhibited root production of Russet Burbank (*Figure 1*).

Microarray-based expression analysis

Both cultivars were inoculated either with strain RG6-12, PsJN or with its derivative PsJN-*aiiA*. The gene expression data of inoculated treatments were compared to those of non-inoculated controls. In *Table 1* are the numbers of up- or down- regulated gene in each cultivar. In Russet Bubank a total number of 530 genes were differentially regulated ($P<0.0001$) comprising a higher number of down-regulated genes than up-regulated ones. In Bionta only 209 genes were differentially regulated comprising a higher number of up-regulated genes than down-regulated ones. In both cultivars the treatment with strain RG6-12 had the lowest impact on gene expression change. Each plant genotype showed a rather unique response to strain PsJN-*aiiA*, whereas more genes were up- or down-regulated in both cultivars after inoculation with the wild-type strains. About hundred genes were significantly down-regulated in Russet Burbank, but up-regulated in Bionta due to strain PsJN-*aiiA*.

The expression change was clustered using K means clustering algorithm. In total 6 clusters were obtained which represent the differences between the genotypes and the effect of the AHLs. *Figure 2* shows the mean expression of the clustered genes. In cluster 3 are most genes which were down regulated in Russtet Burbank and up regulated in Bionta. The genes belong to various functional categories like cell rescue and defense and cell cycle/DNA processing. One gene down regulated in Russet Burbank and up regulated in Bionta are a calmodulin like gene, which ware found to be involved in gene silencing in tobacco (ANANDALAKSHMI et al. 2000). The involvement of this gene in the endophytic plant interaction was proved by real time PCR. Several defense related genes were up

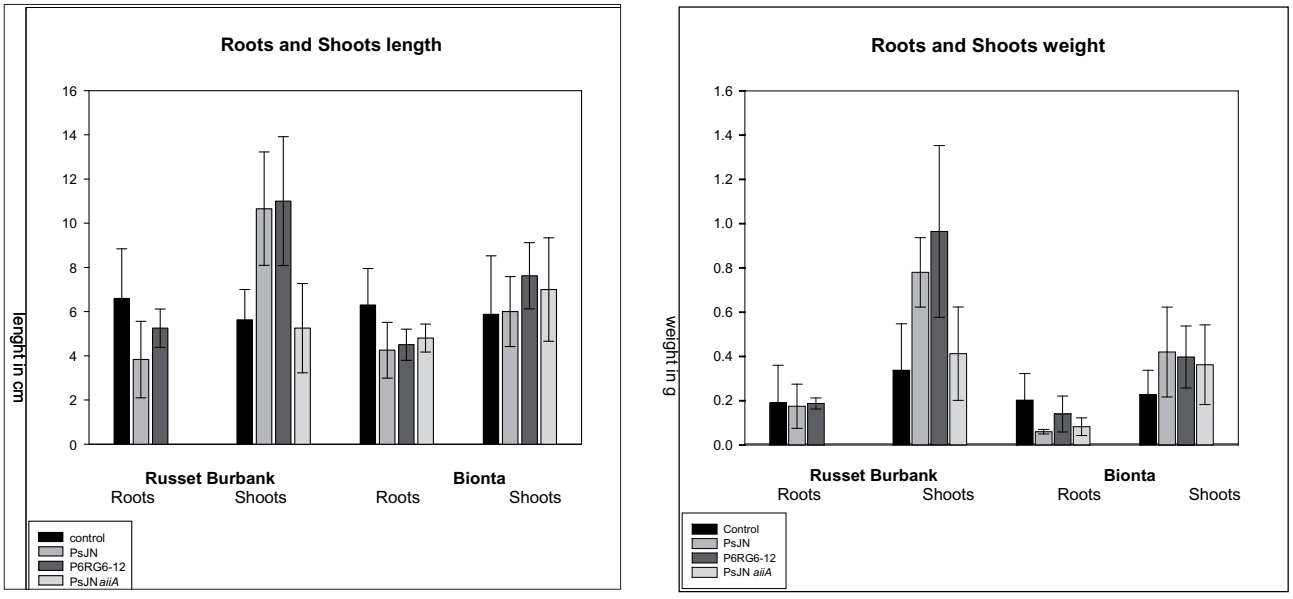

Figure 1: **Root and shoot length (A) and weight (B) of plantlets after inoculation with strains PsJN, RG6-12 and PsJN-*aiiA* or not inoculated**

Table 1: **Number of significantly (P>0.0001) up- or down-regulated genes of Russet Burbank and Bionta in the same treatment, ▲ up-regulated, ▼down-regulated**

	Russet Burbank (▲/▼)	Bionta (▲/▼)	in both cultivars (▲/▼)	Russet Burbank ▲ AND Bionta ▼	Russet Burbank ▼ AND Bionta ▲
RG6-12	33/178	6/13	6/0	0	1
PsJN	81/159	67/33	22/8	0	9
PsJN-*aiiA*	56/225	135/17	4/2	7	106

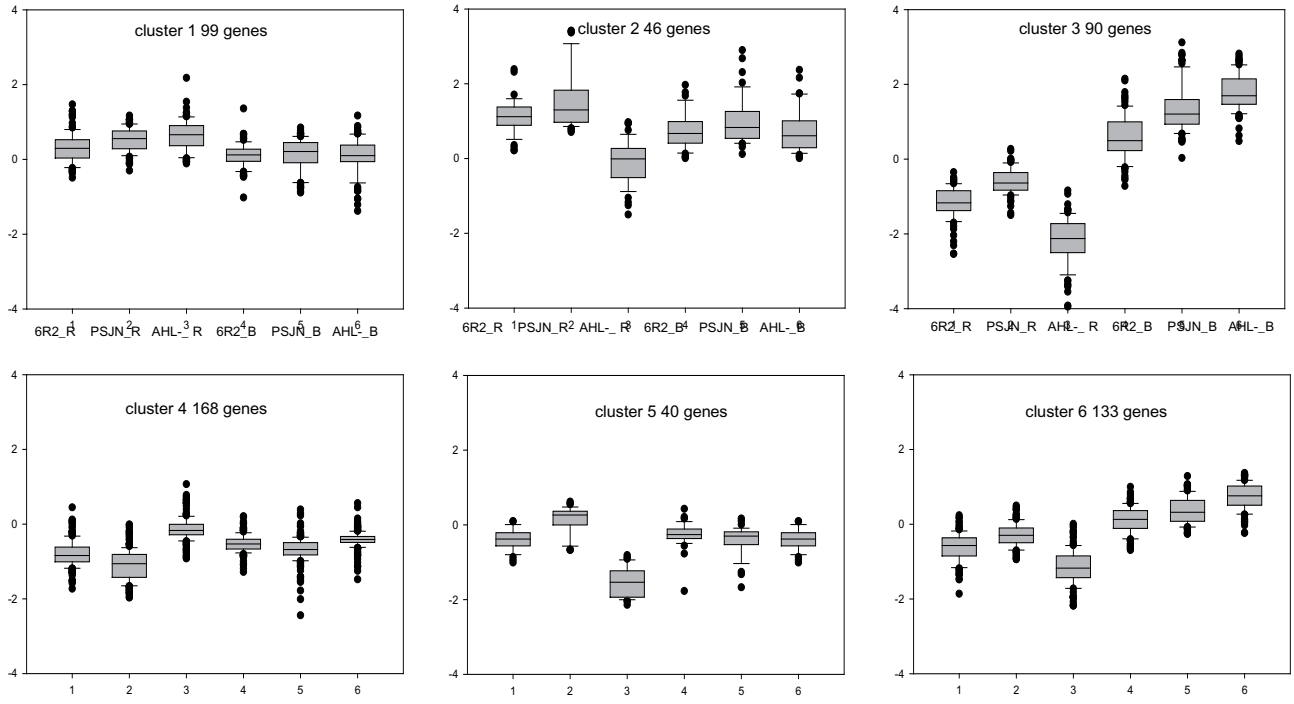

Figure 2: **K means cluster analyse of differential expressed gene among the 6 treatments , 6R2_R (Russet Burbank PR6-12, PsJN_R Russet Burbank PsJN, AHL-_R Russet Burbank PsJN-*aiiA*, 6R2_B (Bionta PR6-12, PsJN_B Bionta PsJN, AHL-_B Bionta PsJN-*aiiA*)**

regulated in Russet Burbank but down regulated in Bionta. To this group belong (cluster 2, *Figure 2*) several proteinase inhibitors, P450 hydroxylase, PR4, lipoxygenase. For this group of genes no significant change was observed in Bionta. Jasmonic acid (JA) is a key signal molecule for these group of genes. Therefore we propose that JA plays a central role in the endophytic plant interaction for plant growth promoting. Several genes involved in photosynthesis were down regulated in Russet Burbank but up regulated in Bionta (cluster 4, *Figure 2*).

The up regulation of defense genes and down regulation of photosynthesis genes by bacteria has been reported previously (CARTIEAUX et al. 2003, ZOU et al. 2005). ZOU et al. (2005) suggested that the down-regulation could be a consequence of a hypersensitive response induced by ROS or localized apoptosis. Down-regulation of photosynthesis and metabolism genes is likely to be only a transient effect needed for the production of other transcripts necessary for the conditions of colonization (CARTIEAUX et al. 2003). In Bionta the up-regulation of defense related genes and down-regulation of photosynthesis genes was also seen but in a less pronounced manner. Our results suggest that in endophyte-plant interactions the beneficial effect may partly depend on the magnitude of the gene expression, but that bacterial colonization is not inhibited by defense mechanisms. In all our experiments, strain PsJN showed efficient endophytic colonization throughout the plant.

Strain AHL-*aiiA* with the quenching of the QS signal OH-C14-AHL had a great impact on the gene expression in both cultivars. In Russet Burbank plant growth was reduced and 166 genes were exclusively down regulated by strain AHL-*aiiA*, whereas only five genes were down regulated in Bionta. Moreover, 106 genes down regulated in Russet Burbank were up regulated in Bionta. Most of these genes are defense-related genes and include a range of transcription factors, late embyogenesis-like genes, phenylalanine ammonia-lyase, Avr9/Cf-9 rapidly elicited proteins, calmodulin-like proteins, a cytochrome P450 encoding gene, a WD repeat 33 protein and stress-induced proteins. A range of PR proteins, ethylene and auxin-sensitive protein, cell wall peroxidase, endochitinase, NDR1, glutaredoxin, WRKY transcription factors and several other wound- and stress-induced genes. Genes specifically up-regulated by strain PsJN-*aiiA* include a MYB111 transcription factor, an expansin precursor, a Kunitz-type trypsin inhibitor, an auxin-inducible protein and jasmonate O-methyltransferase

(cluster 3, 5, 6 *Figure 2*). The highly distinct response of both cultivars to PsJN-*aiiA* suggest that AHLs themselves or micobial metabolites which are produced under the control of quorum sensing play an important role in the interaction with plants and seem to steer beneficial effects.

In conclusion, our study showed that two plant cultivars may respond in a very different and specific manner to growth-promoting bacterial strains and that even highly related microbial genotypes have different effects or induce different responses. Detailed analysis of plant gene expression at several time points after inoculation with wild-type strains as well as with knock-out strains being impaired in the production of AHLs, siderophores, IAA, lipid polysaccharids and other features will give more insight in the interaction of plants with plant growth-promoting bacteria.

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