

Use of beneficial microorganisms for crop improvement

Friederike Trognitz^{1*}, Birgit Mitter¹, Naveed Muhammad¹, Günter Brader¹ and Angela Sessitsch¹

Abstract

Plants live in close relationship with microorganisms and can have a neutral, beneficial or destructive interaction with the plant. Plant beneficial microorganisms stimulate the plant to resist stresses during developmental stages and can occupy niches inside the plant, which are in concurrence with pathogens, and deleterious microorganisms may be used for the biocontrol of weeds. Plant-associated microbial communities are essential for growth parameters like plant nutrition, resistance to biotic and abiotic stresses, plant survival and distribution. *Burkholderia phytofirmans* strain PsJN is a plant growth-promoting bacterium, able to establish both rhizosphere and endophytic populations in a wide variety of plants, including potato, tomato, maize, peat moss and grapevines and stimulates plant growth and plant immune defense in many of its host plants. Several experiments were conducted on maize, pepper and potato plants inoculated with PsJN and the results obtained on its plant growth-promoting effects are reviewed in this report.

Keywords

Biocontrol, endophytes, plant growth promotion

Introduction

In general, agricultural systems are intensive in regard to the requirement of fertilizers, pesticides and water. Sustainable agriculture is based on three main goals: environmental health, economic profitability, and social and economic equity. Microorganisms are important players in a healthy production system and can aid in the development of more sustainable agricultural systems. Like the human body also plants host millions of bacteria living inside or around the plants. Beside pathogens, which can destroy plants completely, many non-pathogenic and mostly beneficial microorganisms are associated with plants. The rhizosphere is a hot spot of microbial diversity and activity due to the secretion of root exudates, which serve as a source of nutrients and attract microorganisms. Root exudates play an important role in signaling and developing microbial communities in different compartments of plants. As different plant species produce different root exudates, they are usually associated with different microbial communities (BERG and SMALLA 2009). Inside plants, endophytes derived from the root environment, thrive on plant compounds and systematically colonize plant tissues (RASCHKE et al. 2006a,b, COMPANT et al. 2010, REINHOLD-HUREK and HUREK 2011). In return, plant-associated microbes may enhance plant growth

by several activities such nutrient acquisition and uptake (N fixation, P and Fe mobilization) as well as synthesis of plant hormones and vitamins. Furthermore, these microbiota improve plant health by out-competing invading or antagonizing or by induction of stress resistance.

The plant-growth promoting *Burkholderia phytofirmans* type strain PsJN (SESSITSCH et al. 2005) isolated from *Glomus vesiculiferum*-infected onion roots (NOWAK et al. 1998) proved to be a potent plant growth promoter (CONN et al. 1997, NOWAK 1998). PsJN developed both endophytic and epiphytic populations on potato, tomato, maize, peat moss and grapevines (FROMMEL et al. 1991, PILLAY and NOWAK 1997, COMPANT et al. 2005, 2008). Biotization with PsJN of *in vitro* potato and other vegetable plants frequently enhanced plant vigor and stress tolerance (LAZAROVITS and NOWAK 1997, NOWAK 1998, NOWAK et al. 2004). Besides stimulating plant growth in a cultivar-dependent manner (BENSALIM et al. 1998, PILLAY and NOWAK 1997), PsJN can induce developmental changes in the host (FROMMEL et al. 1991) that can enhance its water management properties (LAZAROVITS and NOWAK 1997) and also disease resistance (STEWART 1997, SHARMA and NOWAK 1998, AIT BARKA et al. 2002). Plants of specific potato cultivars had larger root systems and developed stolons with tubers earlier than their non-biotized counterparts (DUNBAR 1997). Both nature and magnitude of the plant growth promoting effects vary for unknown reasons, not only across different plant species but also among individual cultivars of one species (CONN et al. 1997, PILLAY and NOWAK 1997, BENSALIM et al. 1998).

Agriculture faces new trends in the last years. Due to the increase in the world population a high demand on food, feed, fiber and renewable raw material is needed, produced on less arable land. Additionally the demand on high quality, healthy and affordable food by the middle-class populations in developing countries is one of the driving force in agriculture production. The requirement of renewable feedstock as alternative energy sources is a new sector in agriculture. To achieve the challenges in agriculture production it is necessary to re-examine many of the existing approaches that include the use of chemical fertilizers, herbicides, fungicides, and insecticides.

Beneficial microorganisms for plant growth promotion

Beneficial microorganisms inhabit the rhizosphere or plant interior and may promote plant growth by several mecha-

¹ Austrian Institute of Technology, Department of Health and Environment, Konrad Lorenz Straße 24, A-3430 TULLN

* Corresponding author: Friederike TROGNITZ, friederike.trognitz@ait.ac.at



nisms. One of the best studied mechanisms is the supply of nutrients like nitrogen, iron and phosphorus (GLICK 2012). But also the level of phytohormones can be modulated by plant growth-promoting bacteria (PGPB). PGPB can influence hormone levels in the plant leading to better stress tolerance in the plant. Furthermore, several phytohormones may be produced by the bacteria like cytokinins, gibberellins, auxins and ethylene.

Burkholderia phytofirmans strain PsJN is one of the best studied plant growth-promoting bacteria. It produces siderophores, auxin and shows 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. ACC deaminase can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in a developing or stressed plant (GLICK 2005). Using PsJN as plant growth-promoting endophyte several crop plants were inoculated in the greenhouse. Table 1 shows the maize cob weight and dry weight of two cultivars inoculated with PsJN. As comparison non-inoculated plants were used. Due to the inoculation about 20% more yield was obtained due to inoculation in the greenhouse. The same effect was also seen for other cultivars like potato and pepper. In potato and maize we observed different responses to inoculation in different cultivars. This indicates that the plant response to inoculation is genetically controlled.

Table 1: Maize yield of two cultivars inoculated with PsJN compared to the non-inoculated control

Cultivar	Maize cob fresh weight (in % to control)	Maize plant dry weight (in % to control)
1	123	125
2	137	130

Not only plant growth is influenced by endophytes but also flowering time, germination rates and resistance to abiotic stress. After inoculation with PsJN, maize flowered 5 to 10 days earlier as compared to control plants. The germination rate of maize inoculated with PsJN was 10% higher and the effect could be seen in 2 different cultivars. These traits have a high agronomic importance and are major breeding goals. To use endophytes under field conditions suitable application technologies have to be developed in order to ensure that endophytes are able to colonize field-grown plants and mediate beneficial effects.

Beneficial microorganism as biocontrol agents

Pest and disease are the major threat in plant production. It is estimated that plant diseases annually cause an 11 to 16% loss of rice, wheat, corn, and potato harvests. For example crop losses due to late blight in potato and its control are estimated to cost worldwide USD 3.25 billion annually, USD

750 million (25%) are being spent on fungicides (GILB, CIP, Lima, Peru; <http://www.cipotato.org/gilb/>). To control diseases in the year 2003 over 219 662 t of plant protection products were used in the EU (FAO). Of these products 49% are used as fungicides and 10% as insecticides. The amounts spent on a global scale reached in 2007/2008 a value of USD 405 billion (ANDREWS et al. 2012).

There is a large body of literature describing potential uses of plant-associated bacteria as agents stimulating plant growth and managing soil and plant health (see review by COMPANT et al. 2005). Bacteria are able to prevent or diminish the effect of deleterious pathogens by direct or indirect manipulation of pathogens. Certain bacteria trigger a phenomenon known as induced systemic resistance (ISR), which is phenotypically similar to systemic induced resistance. The induction of resistance mechanism by the beneficial microorganism brings the plant to a status where it is able to mobilize pathogen defense much faster.

We tested PsJN and its lipopolysaccharides (LPS) on potato leaves for the ability to trigger a faster immune reaction against late blight. For the experiment the true seed potato variety MFII was grown in the greenhouse. One leaf per potato plant was infiltrated with PsJN, its LPS or with buffer as control. Small plantlets were collected and inoculated with a *Phytophthora infestans* suspension or PBS buffer and nitric oxide (NO) and reactive oxygen species (ROS) were measured using online detection methods.

ROS are important signaling molecules that control processes such as pathogen defense, programmed cell death and stomatal functions. Accumulation of ROS in plant cells can result in the formation of the hypersensitive response (HR) and cell wall cross-linking, as well as the induction of the expression of defense-related genes. NO serves as an important signal in plants and animals. Generation of the NO burst is a key feature of the plant defense response following pathogen recognition.

Small leaf stipules from PsJN, LPS and control plants were collected and immersed in *P. infestans* or PBS medium. After 30 min the leaves were transferred to a buffer containing 2',7'-dihydrodichlor-fluorescein-diacetate (H2DCFDA) for ROS detection or 4,5-Diaminofluorescein diacetate (DAF-2 DA) for NO detection. ROS and NO were rapidly increased

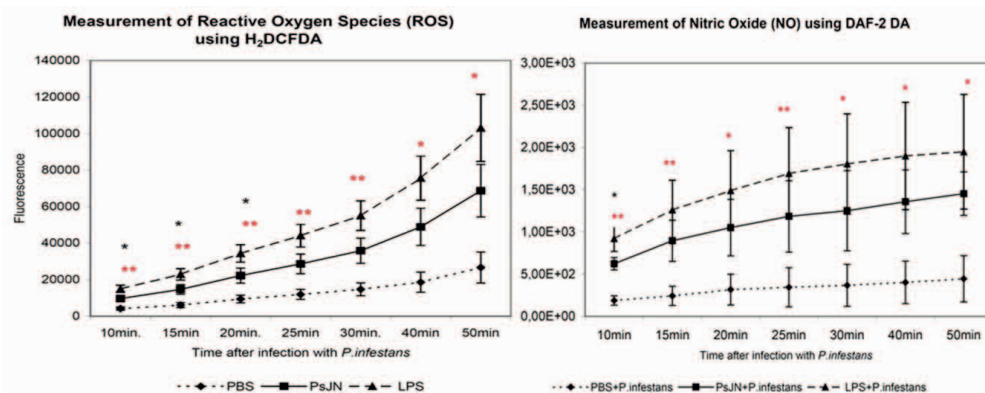


Figure 1: Production of ROS and NO during the interaction with *Phytophthora infestans* in leaves primed with PsJN, LPS or PBS. ROS and NO were rapidly increased after challenging with the pathogen *P. infestans* in the primed leaves with PsJN and LPS compared to the control leaves (PBS). The increase in NO and ROS was faster in the leaves primed with PsJN than in LPS primed leaves.

after challenging with the pathogen *P. infestans* in the primed leaves with PsJN and LPS (Figure 1) compared to control leaves (PBS), but the increase in NO and ROS was faster in leaves primed with PsJN than by LPS treatment. The results showed that PsJN is able to prime the plant in order burst the defense reaction after a pathogen attacks the plant.

Microorganisms as bio-herbicides

In Europe 38% of plant protection products are used for weed control. Weeds produce the highest potential yield loss in agriculture with 34% (OERKE 2006). Studies on bacteria for bio-control against weeds started around 1990. Practical application of bacteria to agriculture could contribute to biological weed management systems that have a lower impact on the environment than conventional systems and reduce the risk of pesticide residuals in the food chain by reducing inputs of herbicides. In the coming years several herbicides will be taken from the market because of their negative effects on soil, water and human health. In the past several plant-associated bacteria have shown negative effects on growth of specific plants (for a review see KREMER and KENNEDY 1996). Among these deleterious bacteria are *Pseudomonas*, *Enterobacter*, *Flavobacterium*, *Citrobacter* and *Achromobacter* strains (KREMER et al. 1990). Metabolites such as auxin and hydrogen cyanide produced by these deleterious bacteria are toxic at high concentrations. Several *Pseudomonas* species and strains showed bio-herbicidal activity against weeds in greenhouses and field tests. For example *P. fluorescens* D7 was active against downy brome (*Bromus tectorum*), *P. fluorescens* LS102 and LS174 against leafy spurge (*Euphorbia esula*) and *P. fluorescens* BRG100 against green foxtail (*Setaria viridis*) (CALDWELL et al. 2012). Host range tests with several strains active against downy brome showed that the effect of the rhizobacteria and their secondary metabolites are host-specific. Tests conducted on non-target hosts (spring and winter wheat) showed no or limited detrimental effects on the plant and in some cases improvement of the growth of the non-target plant was observed (see review by BOYETCHKO 1997).

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