Biotic Stress Relief on Plants in Hydroponic Systems

M. Woitke and W.H. Schnitzler
Center of Life Science Weihenstephan, Technische Universität München
Chair of Vegetable Science - Quality of Vegetal Foodstuff, Freising
Germany

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Abstract

Soilless cultivation techniques or hydroponic growing systems are designed to provide plants with best growing conditions in order to achieve optimum yield. But even under these conditions stress may occur on plants grown in soil as well as soilless, although at different extent, in shorter or longer periods, and more severe and pervasive. Stress can be caused by temperature (heat and cold), too much or too little nutrient supply, drought, salinity, pathogens, but also by insufficient or excessive light and oxygen or carbon dioxide. Therefore, the introduction of so-called beneficial microorganisms (BMO) into different growing systems may prevent either prophylactically or moderate stressing situations. The advantage of applying BMO in controlled growing systems such as hydroponics or soilless can improve efficiency and prolonged activity of BMO due to the lack of competition occurring in soil. In this paper the focus will be on the free-living and more loosely attached root bound bacteria of the group of plant growth promoting rhizobacteria (PGPRs). The other well-known symbiosis of plants and rhizobia, the arbuscular mycorrhiza fungi (AMF) will not discussed here, although both groups are important for biotic stress relief.

INTRODUCTION

Using plant growth promoting rhizobacteria (PGPRs) in agriculture for supporting crop growth via direct effect of growth promotion and indirect effects of biocontrol has already a 100 year long tradition. PGPRs are free-living microorganisms by beneficially colonizing plant root surface and sometimes endophytic (Kloepper et al., 2004). A bulk of knowledge is emerging and available of how these microorganisms interact with their hosts due to more sophisticated new analytical tools in microbiology, biochemistry and molecular biology, leading to gained acceptance of PGPR over the last decade. Several possible mechanisms have been proposed for their effects summarized in Figure1. They include the direct suppression of plant pathogenetic diseases (Smith et al., 1999), the exclusion of less beneficial or pathogenous microorganisms from the root surrounding by competition (Dekkers et al., 1998), the enhancement of the release of limited available nutrients from the soil matrix (Nautiyal et al., 2000, Richardson et al., 2001b, Idriss et al., 2002), the stimulation of host plant disease defense reaction by ISR (induced systemic resistance) via direct plant-microbe interactions (Reddy et al., 1999), and the release of plant-growth regulating substances, i.e. plant hormones (Steenhoudt and Vanderleyden, 2000).

Therefore, the identification, selection and application of suitable beneficial micro-organisms can increase the options to deal with growing problems (Kilian et al., 2000), and additionally are environmentally sound. This way, PGPRs offer great promise for agronomic applications. Nevertheless the selection of adequate species or strains for a specific crop, the determination of suitable conditions for the inoculation of beneficial strains for adequate competitiveness under field conditions remain problematic (Persello-Cartieaux et al., 2003). The same is with maintaining biocontrol agents (Schroth and Hancock 1982, Kloepper, Lifshitz and Zabloticzv 1989).

Open questions are still how best to engineer the beneficial relationship between PGPRs and crop species to become more reliable, the way that the benefits are reproducible and calculable for the grower? Such difficulties will be to overcome easier.
by applying these microorganisms in more controlled environments such as soilless or hydroponic growing systems to provide better possibilities for maintaining stable or high population densities of the BMO over longer time.

The amount of publications dealing with this subject is growing rapidly. Our attempt to describe the main properties of PGPRs should be seen as a compacted extraction with focus on hydroponic applications. For more detailed and specific information see recently published reviews of Whipps 2003, Persello Cartieux 2003, de Kroon and Visser, 2003, Dobbelaere et al., 2003, Kloeper et al., 2004, Lucy et al., 2004.

STRESS RELEASE AND BENEFICIAL EFFECTS OF PGPRs

Major categories of PGPRs are seen as biocontrollers and growth promoters whose effects can be summarized and divided into directly and indirectly benefiting effects towards the host plants (Bashan and Holguin 1998).

Direct effects – growth promotion: The production of plant growth regulators (hormones) has been demonstrated manifold (Glick, 1995). Its consequence is an improved growth of the host plant resulting in enhanced nutrient uptake by better root (i.e., large production of root hairs and lateral roots) and shoot growth (Persello-Cartieaux et al., 2003, own observations in Fig. 2 and Fig. 3) as a consequence and ability of the PGPRs to produce auxins (IAA, indoleacetic acid), cytokinins and by lowering of plant ethylene levels (Arshad and Frankenberger, 1991; Serdyuk et al., 1995; Xie et al., 1996; Idriss et al., 2004). Additionally, the production of even small amounts of gibberellin and jasmonate-like substances were reported (Persello-Cartieaux et al., 2003). Most recently, bacterial produced volatiles have been shown to trigger plant growth enhancement (Ryu et al., 2004).

The provision of bio available phosphorus for plant uptake (Idriss et al., 2002), nitrogen fixation in some genera and a supporting function for the N-fixation by rhizobia (Zang et al., 1996; Bai et al., 2003) as well as the sequestration of iron through siderophores for plant use (O’Sullivan and O’Gara, 1992) count for other notably direct effects.

Indirect effects – biocontrol: These include the production of antibiotics against pathogenetic bacteria, the synthesis of fungal cell wall-lysing enzymes, which are best understood and most investigated facets of PGPRs (Whipps, 2001). The reduction of iron available to phytopathogens by chelating siderophores helps to suppress their growth and multiplication (Loper and Henkels, 1999). Recent studies have shown that the iron nutrition of the plant influences the rhizosphere microbial community structure (Yang and Crowley, 2000). The competition with other soil microorganisms for substrates (Nelson and Hsu, 1994) is a principal factor of importance as well when it comes to their successful suppression.

Another field of rapidly growing attraction is the known ability of PGPRs to induce plant resistance (ISR), which means the activation of a lasting resistance (host plant physical or chemical barriers) to pathogens by biotic or abiotic agents (Van Loon et al., 1998; Zhang et al., 2004; Kloeper et al., in press).

PREREQUISITES AND LIMITATIONS FOR BENEFICIAL INTERRELATIONSHIPS BETWEEN PGPRs AND PLANTS

Many bacteria, even strains within species are often highly adapted to single host plant species, therefore a broader application to different crop species is not necessarily profitable. Comparisons between natural colonizers of Arabidopsis and wheat showed that only the A. thaliana colonizer succeeded when both species were applied to Arabidopsis (Persello-Cartieaux, 2001). But successful root colonization is a prerequisite for succesful applications (Chin –A-Woeng et al., 2000).

Furthermore, many bacteria are weak competitors (Grosch et al., 1996), therefore their effectiveness is highly depending on starting inoculation densities (Bull et al., 1991), microbial communities and competitors (Glick et al., 1999). Even the status of the plant itself influences the balance and the efficacy of the bacterial mode of action. Other
preconditions strongly affecting the single bacterium species or strain can be of abiotic nature: soil pH, temperature, fertilizer and nutrient status of the soil (De Freitas and Germida, 1990). Therefore, more general recommendations strive to use naturally co-occurring BMOs with their host plant. Another method to find suitable BMOs for specific purposes, for example saline growing conditions, is to search for them in such environments by isolation of suitable species with the analysis of positive interaction with the crop.

Successful root colonization by the BMOs and subsequently desired impacts often depends on the initial bacteria inoculum density threshold. This suggests the role of a quorum sensing system in plant-rhizobacteria interactions, whereas the perception of eubacterial flagellin peptides allows the plant to sense the presence of a broad range of microbes (Alström, 1991; Leeman et al. 1995; Felix et al., 1999). As bacterial plant growth promotion can be mediated by additive mechanisms (e.g. siderophore production and ISR), it is tempting to speculate that plants also have developed many strategies to perceive and select their microbial partner (Persello-Cartieaux, 2003).

Under the same category of density dependence in vitro measures of diverse reactions can be correlated to a specific release of, for example, plant hormones or antibiotic active substances, which either cannot be observed in vivo or yield contradictory results.

Looking at the plant hormone auxin (IAA), most notable is for better root development, but also more controlled in vivo experiments show reduced growth of roots due to excessive inoculation densities of IAA-producing strains (Persello-Cartieaux et al., 2001). In another experiment a significant relation between the distance of bacterial inocula to the roots of the host plant was found. Inocula located 2 cm from the plant roots suppressed their growth (leaf area and fresh weight), whereas inocula at a 4 and 6 cm distance increased growth. The responsible substances (‘hormones’) were described diffusible and their efficacy concentration dependent (Ryu et al., in press). In another case, IAA production triggered the ethylene production in the host plant which caused a growth set back (Xie et al., 1996). In own experiments we found in 7 crop species a clear reduction in shoot development at the cost of root structure at the beginning, and later on a deleterious effect for the whole plant development itself, when *Bacillus subtilis* (FZB24) was introduced to 3 weeks old seedlings in sterilized sand at a concentration of $10^9$ cfu (Fig. 4, Fig. 5). Although this relationship of density dependent efficiency is well known and yields high impact, it is has rarely been demonstrated or considered (Yan et al. 2004).

**APPLICATIONS OF PGPRs IN HYDROPONICS**

Main applications of PGPRs are in agriculture and horticulture, less but increasing in forestry and phytoremediation (Lucy et al., 2004). Several PGPR formulations are currently available as commercial products for agricultural production separated into two groups of biofertilizer and biocontrol products. The first category often contains mixtures of several bacteria, the latter often single species. However, little effort is made to understand whether these mixtures of PGPRs are really preferable over single species, and much work has to be done to elucidate synergistic effects (Kloeper et al., 2004). Lucy et al. (2004) gave an overview of bacterial species used and tested over the last 30 years, indicating mainly applications took place in field trials (count for 49 out of 80 examples) over greenhouse (17/80) and soilless (3/80). This suggests that the application is aimed for broad and prophylactic effectiveness.

Other favourite applications are the pre-treatment or hardening of the young plants with PGPRs prior to transplanting into the field for better starting conditions, ISR induction and therefore better protection, which is mentioned to last often over the entire cultivation period with long lasting protection and benefit (Vavrina, 1999, Kokalis-Burelle et al., 2002).

The effectiveness and impact of PGPRs to benefit their hosts is often specific. This fact invited their use in more controlled systems such as hydroponics, which will
allow a better input-output relationship as could be expected from application in soils. If compared to the soil, hydroponics offers the opportunity to control microbial communities and nutrient availability. The success of stress relief can be closer attributed to cause-effect relationship of the single PGP-bacterium. Even the longevity of the beneficial organisms at desired densities can be maintained over a longer period or easier adjusted. When detailed information is available on single bacterial traits and functions for example for salinity stress release, their application in controlled systems can be recommended for higher effectiveness.

CONCLUSIONS
PGRPs have shown to cause beneficial and positive effects on plant performance, when plants were treated at the right time, with the right amount and under the right environmental situation. These are the major interactions for likely benefits growers may expect from such treatments. The better a growing system is controlled the more the peculiarities of this relation can be used for well-aimed purposes. Therefore, such relationships must be considered in detail, with more research directed towards PGPR-plant interactions to avoid such negative effects such as too high spore densities.

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Figures

Fig. 1. Main effects of plant growth promoting rhizobacteria (PGPRs), which benefit plant growth.

![Diagram showing main effects of plant growth promoting rhizobacteria (PGPRs)]

Fig. 2. Root fresh weight, root length and root surface per plant of juvenile tomatoes grown in perlite before transplanting. Plants were inoculated with *Bacillus subtilis* only once at true 4 leaf stage with a $10^8$ cfu/ml spore solution. Root parameters clearly indicate that uptake of nutrients from soil would be favoured by the inoculation with the microorganism. (Columns indicate means, error bars ± S.D.)

![Graphs showing root parameters: fresh weight, length, and surface per plant]
Fig. 3. Concentrations of nitrogen (top) and sodium (bottom) in weekly newly developed shoots of tomatoes grown under different EC levels in soilless culture using perlite as a substrate. Under highest salinity levels the uptake of nitrogen as well as of sodium was highest when plants were inoculated with *Bacillus subtilis* (7.5 + Bs). The effect was more pronounced at the beginning of the experiment (5.5.03) and decreased with time (24.06.03). The most likely explanation for the increased uptake is a larger root system, which enables the plant to increase uptake (cf. Fig. 2). (Columns indicate means, error bars ± S.D.)

Fig. 4. Left side: Performance of shoot and roots of 9 week old *Cucurbita filiciformis* (top) and *Raphanus sativus* (bottom) plants inoculated with *Bacillus subtilis* FZB24 (10^9 cfu/ml, two weeks after germination). Right side: Shoot growth of the control plants (left) is significantly favoured over the root development and vice versa. Plants were grown in sterilized sand and nutritioned with fertilizing solution (EC 2).
Fig. 5. Leaf area, leaf fresh weight, root fresh weight and root/shoot fresh weight ratio of eight vegetable species out of 7 plant families inoculated once with *Bacillus subtilis* 10⁹ cfu/ml spore solution during seedling stage (2 weeks after germination). Plants were grown in sterilized sand and with nutrient solution. Root development of inoculated plants is dramatically favoured over shoot development. Later on, plant faced deleterious growth set back possibly due to too high inoculation densities at the seedling stage. R.c. - *Rucola coltivata* (Brassicaceae), V.l. – *Valerianella locusta* (Valerianellaceae), L.s. - *Lactuca sativa* (Asteraceae), O.b. – *Ocimum basilicum* (Lamiaceae), S.o. – *Spinacia oleracea* (Chenopodiaceae), C.f. – *Cucurbita filiciformis* (Cucurbitaceae), R.s. – *Raphanus sativus* (Brassicaceae), B.o. – *Brassica oleracea* var. gongylodes (Brassicaceae). (Columns indicate means, error bars ± S.D.)