Title
Influence of associative bacteria on root morphology of Arabidopsis plants in dependence on the supplied nitrogen form

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Introduction

Plant growth promoting rhizobacteria (PGPR) colonize the rhizosphere and live in close association with the roots of many crop species, such as wheat or barley. For several decades field trials have been undertaken to investigate the mechanisms of plant growth promotion by these rhizosphere bacteria, but until today these mechanisms remain poorly understood (Okon and Labandera-Gonzales 1994). Besides the fixation of atmospheric nitrogen and release of mineral nitrogen to plant roots, PGPR might produce and release phytohormones (Dobbelaere et al. 1999). However, the complex system of factors influencing the plant-bacterial association might be the reason for the low reproducibility of beneficial effects exerted by inoculated bacteria in field trials. The concentration of the inoculated bacteria or their growth phase at harvest might affect the growth promotion by the bacteria, while the plant species and their nutrient supply might affect the responsiveness of the plant partner. Among these factors that affect the efficiency of the bacterial association, the use of different nitrogen forms has received little attention. It was therefore the aim of this study to investigate whether root growth promotion by PGPR depends on the presence or availability of certain nitrogen forms and concentrations, different bacteria concentrations and phases of growth, and to investigate mechanisms which are responsible the positive effect of inoculation by bacteria.

Methods

*Arabidopsis thaliana* (Col-0) seeds were germinated under sterile conditions on agar plates containing ½ MS medium. After one week, plants were transferred to square plates and continued to grow under different nitrogen supplies for 18 days in the absence or presence of bacteria. At harvest the dry weight of roots and shoots was measured. Plant roots were transferred to transparent slides and roots were scanned to analyze root morphology. The total root length, the total lateral root length and the length of the primary roots were determined using the WinRhizo software.

Roots were inoculated with either *Raoultella terrigena* or *Azospirillum brasilense*. *Raoultella terrigena* was grown in an overnight culture of YEP medium, *Azospirillum brasilense* in L* medium. In a first culture, bacteria were grown until the desired OD was reached. The bacteria were centrifuged and washed two times with phosphate buffer. After each washing step the bacteria were centrifuged again. For inoculation, the bacterial pellet was resolved in double distilled sterile water. A concentration of 10⁵ CFU ml⁻¹ was dropped in the sterile and warm medium before pouring the plates.

Results

*Arabidopsis thaliana* plants inoculated with *Raoultella terrigena* and cultivated under axenic conditions on agar plates showed higher dry weights of shoots and roots, and the root morphology was influenced positively in dependence on the concentration and growth phase of the inoculated bacteria. Growth promotion was further dependent on the height of nitrogen supply and the nitrogen form used to supply the growth medium. Inoculated plants grown on ammonium or ammonium with 10 % nitrate led to most positive effects, while plants with an exclusive supply of nitrate grew better and did not respond positively to inoculation. No positive growth effects were observed in compartimented plates, in which bacteria were separated from the control plants. First analyses of phytohormones indicate a possible release of phytohormones in the presence of bacteria.
Discussion

A positive response of *Arabidopsis* plants to *Raoultella* inoculation was achieved when plants were cultured on ammonium or ammonium with an addition of 10% nitrate, whereas non-inoculated control plants suffered from ammonium-induced growth depression. Thus, *Raoultella* inoculation helped the plants to overcome ammonium toxicity. The mechanisms behind this beneficial action of *Raoultella* and their dependence on ammonium are currently being investigated.

Literature
