Colonization and growth promotion of non-legumes by *Rhizobium* bacteria

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ABSTRACT

Rhizobium leguminosarum bv. *trifolii* strain R39, isolated from red clover nodules, in field experiments on loamy sand, stimulated not only the growth of red clover but also the growth of maize, spring wheat, spring barley and oil radish. After seed inoculation, this strain colonized the rhizosphere of these plants and migrated to nearby non-inoculated spontaneous weed plants. Following crop harvest the *Rhizobium* strain was able to re-establish in the rhizosphere of plants subsequently grown in the same plots. Microbiological and electron microscopical investigations on maize and wheat showed, that R39 had colonized the rhizoplane of the active growing roots [zone of emerging laterals and root tip mucigel]. The production of phytoeffective metabolites by strain 39 may be important for the stimulation of root growth and nutrient uptake by plants.

Introduction

The aim of our research was to screen for plant growth stimulation by *Rhizobium* bacteria in temperate regions.
We have addressed the following questions:

- Are the growth promoting effects of *Rhizobium* specific for crop and microorganism species as well as soil types?
- Which characteristics of microbial metabolism and colonization are of special importance for plant growth stimulation?

The importance of the *Rhizobium* legume symbiosis is well known. In field experiments on loamy sand and sandy loam, we selected, as a first step, *Rhizobium* strains which stimulated the growth of alfalfa and red clover by about 10%. *Rhizobium* spp. are also able to colonize the rhizosphere of cereals [8] stimulating the nitrogenase activity and/or phytohormone production of native rhizosphere microorganisms [6] and promoting the growth of wheat [5]. We therefore analysed the capability of selected *Rhizobium* bacteria to stimulate the growth of gramineae and brassicaceae and the mode of colonization of these plants.

Materials and methods

Fourteen *Rhizobium* strains, which repeatedly stimulated the growth of alfalfa and clover in field experiments, were investigated for their ability to stimulate the growth of maize, wheat, rape and mustard after seed inoculation with peat inoculum (10^8 cfu/g) in pot and field experiments on loamy sand and sandy loam. The metabolism (nitrogenase activity,

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phytohormone production, antagonism of pathogens, pectinase, cellulase, and nitrate reductase activity) of the most effective strain was analysed.

Analysis of the colonization of *Rhizobium* strain R39 on these plants in pot and field experiments was carried out using rifamipicin-resistant mutants and monospecific polyclonal antisera [4, 7, 9, 10]. The identity of the reisolates were tested in qualitative ELISA assays with monospecific polyclonal antisera [7]. Internal root colonization was also estimated by culture of excised tissues of surface-sterilized roots. Electron microscopic investigation of the colonization was made with axenic hydroponic cultures [4, 10].

Results

Plant growth promotion

Only one of 14 strains tested that stimulated the growth of clover or alfalfa also stimulated the growth of non-legumes. In greenhouse experiments, *R. leguminosarum* bv. *trifolii* R39, isolated from red clover nodules, promoted the shoot growth of wheat, maize, oil radish, rape and mustard about 19 to 33 % in comparison with non-inoculated controls. In field experiments on loamy sand seed inoculations with peat formulations of strain R39 significantly and consistently stimulated the growth of red clover, clover-grass-mixtures (7 %), maize (10 %), spring wheat 8 %), spring barley (16 %) and oil radish (21 %)[1, 2, 3]. On sandy loam the effects on non-legumes were not significant. Also, not all maize varieties reacted with a growth stimulation to *Rhizobium* inoculation [2].

In field experiments both the uptake of nitrogen [15-16 %$^{+}$], phosphorus [16-23 %$^{+}$], potassium [21-30 %$^{+}$] in the juvenile stage of maize, and the yield of shoots [13-16 %$^{+}$] and roots [10-22 %$^{+}$] was significant stimulated [2].

Metabolism

Investigation of the metabolism of the *Rhizobium* strain R39 in pure culture showed no nitrogenase activity, but activity of nitrate reductase and production of phytohormones (cytokinin, auxin) was detected. The bacterium also was able to metabolize pectate and cellulose. This strain showed in vitro antagonistic activity against soil-borne plant root pathogens (*Fusarium* spp., *Rhizoctonia* solani, *Helminthosporium sativum* and *Gaeumannomyces graminis*).

In sterile hydroponic cultures (without N) and in pot experiments with loamy sand *Rhizobium* strain R39 stimulated nitrogenase activity on clover but not on wheat. In tubes with carboxymethylcellulose without plants, R39 coinoculated with rhizosphere microorganisms significantly stimulated nitrogenase activity (175 %). In axenic hydroponic cultures with maize, R39 also increased also the auxin content of the culture medium (131%).

Colonization of the plants

Prerequisites for plant growth promotion include not only phytoeffective metabolites by the bacteria, but also survival and establishment of the inoculated strains in the rhizosphere in competition with the native microbial flora. Since young plant roots are sparsely colonized by microorganisms, it is possible to establish a population of selected plant growth-promoting microorganisms via inoculation.

After seed inoculation with peat inoculant, *Rhizobium* strain R39 colonized the roots of gramineae (wheat, maize), leguminoseae (pea, alfalfa), brassicaceae (rape) and chenopodiaceae (sugar beet) when grown in pot experiments with loamy sand [4]. The strain was able to grow along the growing roots to the tip. After shoot inoculation with a bacterial suspension only few bacteria survived in the phyllosphere. Bacteria inoculated to the seed were never reisolated from the phyllosphere.

In field experiments under temperate weather conditions, R39 colonized the rhizosphere (3.0-6.0 log cfu $\cdot$ g^{-1} root of legumes (pea, lupin) as well as of non legumes (maize, wheat and rape)[10]. Moreover, R39 also colonized non-inoculated spontaneous weed plants (*Amaranthus, Echinochloa*) at distance of 40 cm from the inoculated plants. Soil from these field experiments was collected after harvest of the inoculated plants. Non-inoculated maize, pea, lupin and two weeds (*Amaranthus retroflescus, Echinochloa crus-galli*) were subsequently grown in this soil in the greenhouse and were also colonized with R39 (3.0-5.2 log cfu $\cdot$ g^{-1} root)[11]. The *Rhizobium* strain was able to re-establish in the rhizosphere of these plants, even after dry storage of the soil for a period up to 12 month. Highest cell numbers were detected in legumes. Microbial analyses of surface sterilized roots indicated colonization of tissues of the root tip and in the zone of emerging laterals of lupin, broad bean, wheat and maize [4]. With monospecific polyclonal antisera, colonization of R39 in the inner root tissue of maize was detected occasionally. R39 were detected in lysed cells of the root cortex as well as in intercellular spaces of central root cylinder cells [7].

Electron microscopic observations indicated that R39 did not colonize the intercellular spaces of the living cortex tissue or in the xylem of pea, lupin, maize and wheat grown in hydroponic cultures [4, 9]. On these plants, colonization by R39 was observed only on the rhizoplane, especially on active growing arease of the root (the zone of emerging laterals, the root tip mucigel, and the root hair zone). *Rhizobium* attached by filbrillous structures in a polar orientation to the root cell wall, but free microcolonies were also observed. After coinoculation, both strains R39 and the *Pseudomonas fluorescens* strain PsIA12 were able to survive in the same root zone [4].

Conclusions

- In temperate regions selected *Rhizobium* bacteria stimulated the growth of legumes as well as of non-legumes [gramineae cruciferes] in field experiments on loamy sand.
- The *Rhizobium leguminosarum* bv. *trifolii* strain R39, isolated from red clover nodules, colonized the rhizosphere of maize, rape, lupin and pea during plant growth and was able to re-establish in the rhizosphere of subsequent plants.
- The capacity to store poly-ß-hydroxybutyrate as carbon reserve may enable these bacteria to provide energy after dormancy and to re-establish high population numbers as soon as the conditions are favourable again.
- There was no relation between the amount of bacterial colonization in the rhizosphere and the growth stimulation, both *Rhizobium* spp. and *Pseudomonas* spp. were able to survive in the same root zone in close proximity.
- The colonization of active growing root parts [cortex ruptures of emerging laterals, root tip mucigel, root hairzone] and the production of phytoeffective metabolites is important for the root growth and the nutrient uptake of the plants.
- The importance of plant growth promotion by factors such as phytohormone production, N_2 fixation, antagonism may vary due to multiple interactions between inoculated bacteria, native microflora, crops and other ecological factors. More information about these interactions are nessesary.

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